NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for FURAN

FINAL
MARCH 1999

Prepared for

the November 18-19, 1996,
Meeting of the Report on Carcinogens Subcommittee
of the Board of Scientific Counselors

Prepared by

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NTP Report on Carcinogens Listing for Furan

Carcinogenicity

Furan is *reasonably anticipated to be a human carcinogen* based on evidence of malignant tumor formation at multiple tissue sites in multiple species of experimental animals (reviewed in IARC, 1995).

When administered by gavage, furan induced an increase in the incidence of hepatic cholangiocarcinoma, hepatocellular adenoma, hepatocellular carcinoma, and mononuclear cell leukemia in male and female rats treated for up to 2 years (NTP, 1993). Gavage administration of furan to male rats for 9, 12, or 13 months resulted in high incidences of cholangiocarcinoma by 16 months after cessation of treatment (Maronpot et al., 1991; Elmore and Sirica, 1993). When administered by gavage, furan induced a dose-dependent increase in the incidence of hepatocellular adenoma and carcinoma and benign pheochromocytoma in male and female mice treated for up to 2 years (NTP, 1993).

There are no adequate data available to evaluate the carcinogenicity of furan in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

In bacteria, furan induced gene mutations in *Salmonella typhimiurium* strain TA100 (Lee et al., 1994) and in *Escherichia coli* containing bacteriophage T7 (Ronto et al., 1992), but not in *Salmonella typhimurium* strains TA98 (Lee et al., 1994), TA1535, and TA1537 (Mortelmans et al., 1986). In *Drosophila melanogaster*, it did not induce gene mutations (Foureman et al., 1994). In mammalian *in vitro* systems, it induced gene mutations in mouse lymphoma cells (McGregor et al., 1988), sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells (NTP, 1993), and chromosomal damage in CHO cells with an exogenous metabolic activation system (NTP, 1993; Stich et al., 1981; cited by IARC, 1995), but it did not induce DNA damage in mouse or rat hepatocytes (Wilson et al., 1992; NTP, 1993). In mammalian *in vivo* systems, furan induced chromosomal aberrations in bone marrow of mice (NTP, 1993), but did not induce DNA damage in bone marrow or hepatocytes of mice (Wilson et al., 1992; NTP, 1993) or hepatocytes of rats (Wilson et al., 1992).

A current hypothesis for the mechanism of furan-induced carcinogenesis is metabolic activation of furan by cytochrome P-450 to a reactive and cytotoxic intermediate that stimulates cell replication, increasing the likelihood of tumor induction (Chen et al., 1995; Kedderis et al., 1993). The postulated reactive metabolite is *cis*-2-butene-1,4-dial, which was recently characterized as a furan metabolite by Chen et al. (1995). This reactive metabolite probably explains furan's binding reactivity with proteins both *in vitro* (uninduced and induced male rat liver microsomes) and *in vivo* (male rat liver protein) in biological systems (Burka et al., 1991; Parmar and Burka, 1993). Furan metabolites may react with DNA, but Burka et al. (1991) did not detect any radiotracer in DNA from livers of rats treated with [^{14}C]furan.

No data are available that would suggest that the mechanisms thought to account for tumor induction by furan in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

Furan
[110-00-9]
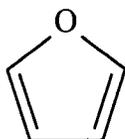
1.1 Chemical Identification

Furan (C_4H_4O, CASRN 110-00-9, mol. wt. = 68.08) is also called:

Furan (8CI, 9CI)	NCI-C56202
Axole	Oxacyclopentadiene
Divinylene oxide	Oxole
1,4-Epoxy-1,3-butadiene	Tetrol
Furfuran	Tetrole

Furan's RCRA waste number is U124, and in shipping, its UN number is 2389.

1.2 Physical-Chemical Properties

Property	Information	Reference
Molecular Weight	68.08	Budavari (1996)
Color	Clear, colorless	McKillip et al. (1989, cited by IARC, 1995)
Physical State	Liquid	Budavari (1996)
Melting Point, °C	-85.6	Weast and Astle (1980)
Boiling Point at 760 mm, °C	31.36	Budavari (1996)
Density at 20 °C/4 °C, g/mL	0.9514	Weast and Astle (1980)
Odor	Strong ether-like odor	McKillip et al. (1989; cited by IARC, 1995)
Solubility:		
Water at 20 °C	Insoluble	Budavari (1996)
Organic Solvents	Freely soluble in: ethyl alcohol, diethyl ether, acetone, and benzene	Budavari (1996); Weast and Astle (1980)
Partition Coefficients:		
Log octanol/water (Log P)	1.34	Hansch et al. (1995; cited by IARC, 1995)
Vapor pressure at 25 °C, torr	599.9	Driesbach (1961; cited by ISHOW)

1.3 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report include the following:

2-Furyl methyl ketone (2-acetylfuran, $C_6H_6O_2$, MW = 110.11)
cis-2-Butene-1,4-dial (cyclic hydrates under physiological conditions)

2-Furyl methyl ketone is soluble in ethanol and diethyl ether (Weast and Astle, 1980). Spectral data were the only physical-chemical properties found for *cis*-2-butene-1,4-dial (Chen et al., 1995). The structures for the analogues are as follows:

2-Furyl methyl ketone
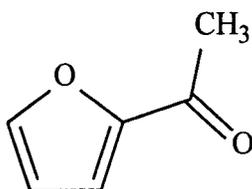
cis-2-Butene-1,4-dial and its cyclic hydrates
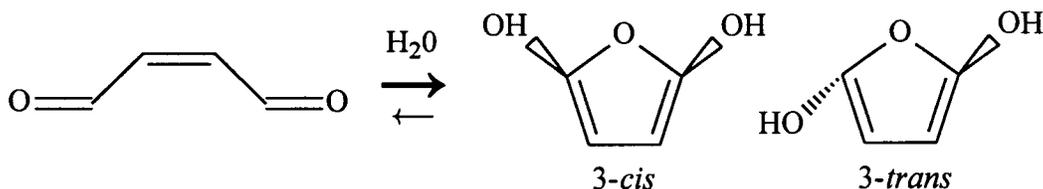
1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Data, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

2.0 HUMAN EXPOSURE

2.1 Production

One company in the United States produces furan (Chemical Information Services, Inc., 1994; cited by IARC, 1995). Commercial production of furan involves decarbonylation of furfural over a palladium/charcoal catalyst (McKillip et al., 1989; cited by IARC, 1995). No data on imports or exports of furan were available. Chem Sources (1996) identified 21 U.S. suppliers.

2.2 Use

Furan is used primarily as an intermediate in the synthesis and production of other organic compounds. Hydrogenation of furan over a nickel catalyst produces high yields of tetrahydrofuran and is a source of commercial tetrahydrofuran (McKillip and Sherman; 1980, and McKillip et al., 1989; both cited by IARC, 1995; NTP, 1991). Furan may also be used as a starting material in the commercial production of thiophene. Furan is also used in the preparation of numerous polymeric compounds. Copolymers of maleic acid and furan form complexes with alkaline earth ions. These complexes are used as alternatives to phosphorus- and nitrogen-containing machine dishwashing products, and detergents used for washing food products and degreasing metals (Kirk-Othmer, 1978; cited by NTP, 1991). A major manufacturer in a mid-1980's product brochure did not mention use of furan in polymer production (OHMTADS Accession Number 8300177, field USS), but stated that ▯Furan polymers▯ are generally produced from furfuryl alcohol. Furan is used in the formation of lacquers and solvent for resins. It is also used in the production of agricultural chemicals (insecticides) and stabilizers and pharmaceuticals (McKillip and Sherman, 1980 and McKillip et al., 1989; both cited by IARC, 1995).

2.3 Environmental Exposure

2.3.1 Environmental Occurrence

In a study conducted by Jarke et al. (1981), furan was detected in the indoor air of homes in the Chicago and Washington, D.C. metropolitan areas, but its concentration (< 100 ppb) was not considered hazardous by the authors.

2.3.2 Environmental Release

Furan was detected in 1 of 63 industrial effluents at a concentration of < 10 μg/L (Perry et al., 1979; cited by IARC, 1995). It was also found at a concentration of 7 ± 4 ppb (μg/L) in aqueous condensate samples from low-temperature gasification of rosebud coal. At a detection limit of 0.1 ppb, furan was not detected in retort water from shale processing *in situ*, in boiler blowdown water from oil-shale processing *in situ*, groundwater or coal water before coal gasification *in situ*, or in water samples obtained during coal gasification in situ (Pellizari et al., 1979).

Furan was detected in a creek in the Niagara River watershed, either in water at a level of 0.1 to 1 ppb or in sediment at a level of 0.5 to 2 ppm. It was not specified in which medium

furan was detected and the concentration of furan was not quantitated (Elder et al., 1981). Qualitative analyses have also identified furan in the Niagara River itself (Howard et al., 1990; cited by IARC, 1995).

Furan is released as a gas-phase component of wood smoke, cigarette smoke, and exhaust gas from diesel and gasoline engines (Howard et al., 1990; cited by IARC, 1995).

In Flanders, a study of nuisance odors showed a concentration of 170 μg furan/m^3 from the emissions of a deep fat frier (Moortgat et al., 1992; cited by IARC, 1995).

2.3.3 Drinking Water and Food Content

Furan has been identified in cooked beef aroma using a headspace sampling procedure (Galt and MacLeod, 1984).

Furan was identified, but not quantitated, using gas chromatography/mass spectrometry (GC/MS) in the breast milk of 1 of 8 lactating women. The women were recruited from hospitals and clinics in Louisiana, New Jersey, and Pennsylvania (Pellizzari et al., 1982).

2.3.4 Consumer Products

The pattern of commercial furan use suggests that minimal exposure to the general public would be expected through contact with products contaminated with furan (NTP, 1991).

In the expired air of cigarette smokers, it has been detected at 0 to 98 μg/h, while in nonsmokers, it has been detected in the breath at 0 to 28 μg/h (Howard et al., 1990; cited by IARC, 1995).

In an attempt to establish a trace organic compound baseline in human respired air, Conkle et al. (1975) analyzed the breaths of 8 male volunteers. The subjects ranged in age from 23 to 47 years, with a median age of 38. Two subjects were moderate cigarette smokers and one was a light cigar smoker. All subjects were either military or civilian employees of the U.S. Air Force School of Aerospace Medicine and were in good physical condition. Samples were collected for a period of approximately 60 minutes. Furan was detected in 6 of 8 subjects. The concentration of furan in expired air ranged from 0.25 to 98.0 μg/h. Both the highest and lowest concentrations were detected in smokers.

2.3.5 Occupational Exposures

The primary route of potential human exposure to furan is inhalation. Since the industrial processes in which furan are used are conducted in closed systems and its volatility requires that furan be handled in closed containers, occupational exposure is limited (NTP, 1991). The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 244 workers were potentially exposed to furan in the workplace (NIOSH, 1976). The National Occupational Exposure Survey (1981-1983) indicated that 35 workers,including 7 women, were potentially exposed to furan (NIOSH, 1984). In one study, furan was detected in waste gases during drying of molassed beet pulp (Oldfield et al., 1979 abstract).

2.4 Regulations

EPA regulates furan under the Resource Conservation and Recovery Act (RCRA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Superfund Amendments and Reauthorization Act (SARA), and the Toxic Substances Control Act (TSCA). EPA has established rules for regulating hazardous spills and for reporting such spills or releases. EPA has also set general threshold amounts, and established requirements for handling and disposal of furan wastes. Furan is regulated as a hazardous constituent of waste under RCRA and is subject to report/recordkeeping requirements under RCRA and SARA. A statutory reportable quantity (RQ) of 1 lb was established for furan, but EPA increased the RQ to 100 lb under CERCLA. The Department of Transportation (DOT) has its own regulations for the transportation of furan in tank cars and tank trucks. OSHA regulates furan under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 148.14(d). Effective 08/08/90. RCRA 3004: Final rule for Waste Specific Prohibitions for Hazardous Waste Injection.	These wastes are prohibited from underground injection at off-site injection facility.
	40 CFR 148.14(f). Effective 11/08/90. RCRA 3004: Final rule for Waste Specific Prohibitions for Hazardous Waste Injection.	These wastes are prohibited from underground injection at off-site injection facility.
	40 CFR 261.33. Promulgated 11/25/80. RCRA 3010: Identification and listing of Hazardous Waste. Designates furan as a hazardous waste subject to recordkeeping and reporting requirements.	Furan has been identified as a primary hazardous material (U124) by its ignitability (I).
	40 CFR 261.33(f). Promulgated 07/01/90. RCRA 3010: Final rule for discarded commercial chemical products, off-specification species, container residues, and spill residues.	Chemical class U wastes and toxic wastes.
	40 CFR 268.10. Promulgated 07/01/91. RCRA 3004: Schedule for land disposal prohibition and establishment of treatment standards. Identifies restricted wastes and concentrations of their hazardous constituents which may not be exceeded.	Restrictions or prohibitions for storage and land disposal of furan to be evaluated by August 8, 1988.
	40 CFR 268.35(a). Effective 01/31/91. RCRA 3004: Technical amendment to the final rule for effective dates of surface disposed wastes (non-soil and debris) regulated in the Land Disposal Restrictions - Comprehensive List.	Waste-specific prohibitions - Third Third wastes. Effective date of prohibition from land disposal was 08/08/90.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 268.35(d). Effective 01/31/91. Revised at 57 FR 47776, 10/20/92. RCRA 3004: Technical amendment to the final rule for effective dates of surface disposed wastes (non-soil and debris) regulated in the Land Disposal Restrictions for mixed radioactive/hazardous wastes.	Effective date of prohibition from land disposal was 05/08/92. Furan is a hazardous component of mixed radioactive/hazardous wastes.
	40 CFR 268.42, Table 2. Effective 01/31/91. Amended through 05/24/93. RCRA: Treatment Standards Expressed as Specific Technologies.	Listing of Technology-Based Standards by RCRA waste code in wastewater as wet air oxidation or chemical/electrolytic oxidation followed by carbon adsorption or incineration. Nonwastewater disposal is by fuel substitution or incineration.
	40 CFR 268, Appendix IV. Effective 01/31/91. RCRA: Land Disposal Restrictions on Organometallic Lab Packs.	Lists hazardous waste by the EPA hazardous waste code number (U124) for disposal in an organometallic lab pack.
	40 CFR 268, Appendix V. Effective 01/31/91. RCRA: Land Disposal Restrictions on Organic Lab Packs.	Lists hazardous waste by the EPA hazardous waste code number (U124) for disposal in an organic lab pack.
	40 CFR 268, Appendix VII, Table 1. Effective 01/31/91. RCRA: Land Disposal Restrictions, Comprehensive List.	Comprehensive listing of the effective dates of surface disposal wastes (non-soil and debris) regulated in the Land Disposal Restrictions (LDRs) and listed by the EPA hazardous waste code number. U124 has an effective date of 08/08/90 in all waste categories.
	56 FR 7134. Promulgated 08/21/91. RCRA: Final rule expanding controls on hazardous waste combustion to regulate air emissions from the burning of hazardous waste in boilers and industrial furnaces.	As a toxic organic compound, emissions testing and health-risk assessment is required for furans at facilities meeting specified criteria where the potential for significant concentrations may exist. Generally, the "furans" meant by these regulations are polychlorinated dibenzofurans (PCDFs).
	40 CFR 302.4, Table 302.4. Promulgated 8/14/89. CERCLA 102(a): List of hazardous substances and reportable quantities.	Final rule established RQ of 100 lbs (45.4 kg) for furan under CERCLA 102(a) when RCRA 3001 established the RQ of 1 lb (0.454 kg).

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 355, Appendices A and B. Promulgated 4/22/87. SARA 302: Final rule establishes a list of extremely hazardous substances, threshold planning quantities, and facility notification responsibilities for state and local emergency response plans.	Threshold planning quantity of 500 lbs (227.0 kg) and reportable quantity of 100 lbs (45.4 kg) were established.
	56 FR 52175. Proposed 10/17/91. SARA 110 and CERCLA 104(i): National Priorities List Hazardous Substances, Toxicological Profiles.	This previously listed substance not included on the Revised Priority List.
	Clean Air Act, Section 112 (r), Risk Management Planning.	Threshold quantity for furan is 5000 lbs (2270 kg).
O S H A	29 CFR 1910.119, Appendix A. Proposed 02/24/92. OSH Act 4, 6, and 8: Final rule listing highly hazardous chemicals, toxics and reactives (mandatory), and their threshold quantities. OSHA Process Safety Management of Highly Hazardous Chemicals.	Threshold quantity for furan is 500 lbs (227 kg).
	29 CFR 1910.1450. Promulgated 1/31/90. Amended 55 FR 12111, 3/30/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	Requires employers to provide employee information and training and to provide Chemical Hygiene Plan.
	55 FR 29150. Proposed 7/17/90. OSH Act: Establishes procedures for process safety management that would protect employees from the hazards of toxicity, fire, or explosion from major industrial accidents.	

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
D O T	49 CFR 172.101. Effective 10/01/91. Amended 58 FR 51531, 10/1/93. DOT: Hazardous Materials Table.	The DOT classifies the materials within as hazardous for the purpose of transportation, setting requirements for the packaging, labelling, and quantity limits aboard passenger aircraft or railcar as 1 L and cargo aircraft only as 30 L. In addition, quantity limitations are also set for stowage aboard vessels.
	49 CFR 172.101, Appendix A, Table 1. Effective 12/31/91. Amended 58 FR 51528, 10/1/93. DOT: List of Hazardous Substances and Reportable Quantities.	Lists materials that are considered hazardous substances under CERCLA with their corresponding reportable quantities (RQs). For furan the RQ is 100 lbs (45.4 kg).
	49 CFR 172.102. Promulgated 10/01/90. Amended 58 FR 51531, 10/1/93. DOT: Special Provisions for Transportation.	Special provisions are given for packaging, labelling, and transportation of hazardous materials in Table 172.101. Flammable liquid label required for furan.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of furan in humans.

4.0 MAMMALIAN CARCINOGENICITY

Full experimental details for the studies described in this section are presented in Table 4-1.

Summary: There is "sufficient evidence" for the carcinogenicity of furan in experimental animals (IARC, 1995). The incidence of hepatocellular adenoma was increased in male and female B6C3F$_1$ mice and in male and female F344/N rats treated for up to 2 years by gavage with furan. The incidence of benign adrenal pheochromocytoma was increased in male and female B6C3F$_1$ mice treated for up to 2 years by gavage with furan. The incidence of hepatocellular carcinoma was increased in male and female B6C3F$_1$ mice and in male, but not female, F344/N rats treated for up to 2 years by gavage with furan. The incidence of cholangiocarcinoma was increased in male and female F344/N rats treated for 9, 15, or 24 months by gavage with furan and in male F344/N rats (females not evaluated) treated for 6, 9, 12, or 13 weeks by gavage with furan and then held for up to 16 months without treatment. The incidence of mononuclear cell leukemia was increased in male and female F344/N rats treated for 2 years by gavage with furan. Doses for these studies ranged from 2 to 30 mg furan per kilogram mean body weight (29 to 441 μmol/kg).

4.1 Mice

There was an increase in the incidences of hepatocellular adenomas in male and female B6C3F$_1$ mice administered 8 or 15 mg furan/kg (117 or 220 μmol/kg) and of hepatocellular carcinoma in males administered 8 or 15 mg/kg and females administered 15 mg/kg by gavage for up to 2 years (adenoma: 33/50 low-dose males and 42/50 high-dose males vs. 20/50 male controls; 31/50 low-dose females and 48/50 high-dose females vs. 5/50 female controls; carcinoma: 32/50 low-dose males and 34/50 high-dose males vs. 7/50 male controls; 27/50 high-dose females vs. 2/50 female controls). Hepatocellular carcinoma was detected in 7/50 low-dose females (this was not statistically significant). The incidence of benign adrenal pheochromocytoma was also significantly increased in low- and high-dose males (6/50 and 10/50 vs. 1/49 controls) and in high-dose females (6/50 vs. 2/50 controls) (NTP, 1993).

4.2 Rats

Male and female F344/N rats were treated by gavage for 9, 15, or 24 months with 2, 4, or 8 mg furan/kg (29, 59, or 117 μmol/kg) per day, 5 days/week. After 9, 15, and 24 months, the incidence of cholangiocarcinoma was significantly increased in all furan-treated rats. After 24 months, the incidence of hepatocellular adenoma was also significantly increased in furan-treated rats administered the mid- or high-dose and the incidence of hepatocellular carcinoma was significantly increased in furan-treated males, but not females, administered the mid- or high-dose. Also after 24 months, the incidence of mononuclear cell leukemia was significantly

increased in furan-treated rats administered the mid- or high-dose (Maronpot et al., 1991; NTP, 1993).

Cholangial or hepatocellular neoplasms were detected in all male F344/N rats treated with 30 mg furan/kg/day (441 μmol/kg/day) by gavage for 13 weeks and then held for 9 or 15 additional months without receiving treatment (cholangiocarcinoma: 0/10, 10/10, and 10/10 rats at 13 weeks, 9 months, and 15 months, respectively; hepatocellular carcinoma: 0/10, 0/10, and 2/10 rats at 13 weeks, 9 months, and 15 months, respectively) (Maronpot et al., 1991; NTP, 1993).

Furan was reported to induce a carcinogenic response in rats in a study conducted by Elmore and Sirica (1993). Young adult F344 male rats were treated with 30 mg furan/kg (441 μmol/kg) by gavage for 9, 12, or 13 weeks. Hepatic adenocarcinomas morphologically consistent with cholangiocarcinomas were present when the rats were sampled 16 months after cessation of treatment. The statistical significance of this finding is unknown because no mention was made of control animals.

TABLE 4-1. Mammalian Carcinogenicity of Furan

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - *Oral Administration*							
58-day-old B6C3F₁	50M, 50F (for both dose levels)	50M, 50F (corn oil alone)	furan, >99% pure	8 or 15 mg/kg bw/day by gavage in corn oil, 5 days/wk (117 or 220 μmol/kg/day)	up to 104 wk	Mice were killed at the end of 2 years. Statistical analyses of incidence were performed using logistic regression tests. **Liver:** Positive (for carcinogenesis) The incidence of hepatocellular adenoma was significantly increased, in a dose-response manner, in furan-treated mice (33/50 low-dose males [p = 0.001], 42/50 high-dose males [p < 0.001], 31/50 low-dose females [p < 0.001], and 48/50 high-dose females [p < 0.001] vs.20/50 male controls and 5/50 female controls). The incidence of hepatocellular carcinoma was significantly increased, in a dose-response manner, in furan-treated males that received either the low or high dose and in furan-treated females that received the high dose (32/50 low-dose males, 34/50 high-dose males, and 27/50 high-dose females vs. 7/50 male controls and 2/50 female controls; p < 0.001). Hepatocellular carcinoma was detected in 7/50 low-dose females (this was not statistically significant). **Adrenal Medulla:** The incidence of benign pheochromocytoma was significantly increased in low- and high-dose males (6/50 [p = 0.032] and 10/50 [p = 0.009] vs. 1/49 controls) and in high-dose females (6/50 vs. 2/50 controls, p = 0.040). Benign pheochromocytoma was detected in 1/50 low-dose females (not significant).	NTP (1993)

TABLE 4-1. Mammalian Carcinogenicity of Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - _Oral Administration_							
51-day-old F344/N	70M, 70F (for each dose level)	70M, 70F (corn oil alone)	furan >99% pure	2, 4, or 8 mg/kg bw/day by gavage in corn oil, 5 days/wk (29, 59, or 117 μmol/kg/day)	9, 15, or 24 mo	Rats were killed after 9, 15, or 24 months. Statistical analyses of incidence were performed using logistic regression tests. Liver: Positive (for carcinogenesis) At the 9-month interim evaluation, the incidence of cholangiocarcinoma was significantly increased in furan-treated rats (5/10 low-dose males [$p \le 0.05$], 7/10 mid-dose males [$p \le 0.01$], and 10/10 high-dose males [$p \le 0.01$] vs. 0/10 male controls; 4/10 low-dose females [$p \le 0.05$], 9/10 mid-dose females [$p \le 0.01$], and 10/10 high-dose females [$p \le 0.01$] vs. 0/10 female controls). At the 15-month interim evaluation, the incidence of cholangiocarcinoma was significantly increased in furan-treated rats (7/9 low-dose males [$p \le 0.01$], 9/9 mid-dose males [$p \le 0.01$], and 6/6 high-dose males [$p \le 0.01$] vs. 0/9 male controls; 9/10 low-dose females [$p \le 0.01$], 9/9 mid-dose females [$p \le 0.01$], and 7/7 high-dose females [$p \le 0.01$] vs. 0/9 female controls). After 2 years, the incidence of cholangiocarcinoma was significantly increased in furan-treated rats (43/50 low-dose males [$p \le 0.001$], 48/50 mid-dose males [$p \le 0.001$], and 49/50 high-dose males [$p \le 0.001$] vs. 0/50 male controls; 49/50 low-dose females [$p \le 0.001$], 50/50 mid-dose females [$p \le 0.001$], and 48/50 high-dose females [$p \le 0.001$] vs.0/50 female controls). After 2 years, the incidence of hepatocellular adenoma was also significantly increased in furan-treated rats administered the mid- and high-doses (18/50 mid-dose males [$p < 0.001$] and 27/50 high-dose males [$p < 0.001$] vs. 1/50 male controls; 4/50 mid-dose females [$p = 0.048$] and 7/50 high-dose females [$p = 0.002$] vs. 0/50 female controls).	Maronpot et al. (1991); NTP (1993)

TABLE 4-1. Mammalian Carcinogenicity of Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
51-day-old F344/N	70M, 70F (for each dose level)	70M, 70F (corn oil alone)	furan >99% pure	2, 4, or 8 mg/kg bw/day by gavage in corn oil, 5 days/wk (29, 59, or 117 μmol/kg/day)	9, 15, or 24 mo	After 2 years, the incidence of hepatocellular carcinoma was significantly increased in furan-treated males, but not females, administered the mid- and high-dose (6/50 mid-dose males [p = 0.009] and 18/50 high-dose males [p < 0.001] vs. 0/50 male controls). The incidences of hepatocellular adenoma and carcinoma were not tabulated for the 9- and 15-month interim evaluations **Blood:** Positive (for mononuclear cell leukemia) After 2 years, the incidence of mononuclear cell leukemia was significantly increased in furan-treated rats administered the mid- or high-dose (17/50 mid-dose males [p = 0.027] and 25/50 high-dose males [p < 0.001] vs. 8/50 male controls; 17/50 mid-dose females [p = 0.034] and 21/50 high-dose females [p = 0.008] vs. 8/50 female controls).	NTP (1993)
47- to 61-day-old F344/N	30M	10M (at 9 mo) 10M (at 15 mo)	furan >99% pure	30 mg/kg bw/day by gavage in corn oil, 5 days/wk (441 μmol/kg bw/day)	13 wk	Rats were treated for 13 weeks and then held for up to 15 additional months without receiving treatment. Groups of 10 rats were evaluated after 13 weeks, 9 months, or 15 months. Statistical analyses of incidence were performed using logistic regression tests. **Liver:** Positive (for carcinogenesis at 9 and 15 months) Early furan-induced liver changes included cholangiofibrosis and cholangiohepatitis. The progressive growth of these cholangial lesions over time, their transplantability, and the development of metastases in some transplant recipients provide biological evidence of the malignant potential of the furan-induced liver changes. Cholangiocarcinoma was detected in 0/10, 10/10, and 10/10 furan-treated rats 13 weeks, 9 months, and 15 months, respectively, after the start of furan treatment. Cholangiocarcinoma was detected in 6/6 furan-treated rats between 9 and 15 months and in 14/14 furan-treated rats after 15 months. Hepatocellular carcinoma was detected in 0/10, 0/10, and 2/10 furan-treated rats 13 weeks, 9 months, and 15 months, respectively, after the start of furan treatment. Hepatocellular carcinoma was detected in 0/6 furan-treated rats between 9 and 15 months and in 4/14 furan-treated rats after 15 months.	Maronpot et al. (1991) NTP (1993)

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TABLE 4-1. Mammalian Carcinogenicity of Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
young adult F344	10M (6-wk exposure) 10M (9 wk) 10M (12 wk) 12M (13 wk)	No controls mentioned in article	furan, 99+% pure	30 mg/kg bw/day by gavage in corn oil, 5 days/wk (441 µmol/kg bw/day)	6, 9, 12, or 13 wk	Animals were observed for up to 16 months. **Liver:** There was a higher incidence of hepatic adenocarcinoma in the right/caudate liver lobe than in the left/median liver lobe of rats treated with furan for 13 weeks (9/10 in right/caudate lobe vs. 3/10 in left median lobe. [It was noted that in these 3 rats, a larger hepatic neoplasm was detected in the region of the right/caudate liver lobe, so that the incidence in the left/median lobe may have reflected intrahepatic spread rather than separate primary neoplasms]). In rats treated with furan for 6, 9, or 12 weeks, 4/9, 6/8, and 5/7, respectively, developed hepatic adenocarcinoma. The location of these tumors (right/caudate or left/median liver lobe) was not described.	Elmore and Sirica (1993)

Abbreviations: bw = body weight; mo = month(s); wk = weeks(s): M = male; F = female

5.0 GENOTOXICITY

Studies of the genotoxic effects of furan are summarized in Table 5-1.

Summary: Furan was found to exhibit genotoxicity in a limited variety of prokaryotic and mammalian *in vitro* and *in vivo* test systems (data limited to IARC, 1995). When tested *in vitro*, furan was found to induce gene mutations in *Salmonella typhimurium* and mouse lymphoma cells; mutational inactivation in bacteriophage T7; sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells; and chromosomal aberrations in CHO cells *in vitro* with metabolic activation and mouse bone marrow *in vivo*. It was negative for induction of sex-linked recessive mutations in *Drosophila melanogaster;* SCE in mouse bone marrow; and unscheduled DNA synthesis (UDS) in both mouse and rat hepatocytes *in vitro* and *in vivo*.

Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*. Information for studies reviewed in IARC (1995) was often limited to qualitative data with information on study design, doses tested, chemical purity, etc., generally not provided.

5.1 Noneukaryotic Systems

As reported by Mortelmans et al. (1986), furan at concentrations up to 3333 μg/plate (0.49 μmol/plate) was negative for the induction of reverse mutations in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without rat or hamster S9 activation using the pre-incubation assay. However, in a plate incorporation study conducted by Lee et al. (1994), furan tested at 0.8 to 100 μmol/plate was reported to induce a positive mutagenic response in strain TA100 both with (LED = 20 μmol/plate) and without S9 (LED = 100 μmol/plate) but not in strain TA98.

Ronto et al. (1989) reported that furan tested in the range from 1 to 10 mg /mL (15,000-150,000 μM) was positive for inactivation of bacteriophage T7 when subsequently plated on *E.* strain B. Furan gave a mutation index (in minutes x mol/L; the reciprocal of the probability of phage inactivation by the unit concentration of the chemical) of 6.8, where values smaller than 8.0 are considered positive.

5.2 Lower Eukaryotic Systems

Foureman et al. (1994; cited by IARC, 1995) concluded that furan was negative for the induction of sex-linked recessive lethal mutations in strain Canton-S males dosed either by feeding at 10,000 ppm (146,908 μmol/kg) or injection at 25,000 ppm (367,269 μmol/kg) and then mated with *Basc* females.

5.3 Mammalian Systems *In Vitro*

5.3.1 Gene Mutations

McGregor et al. (1988; cited by IARC, 1995) reported that furan, at doses ranging from 1139 to 3800 μg/mL (17,000 to 56,000 μM) in the absence of metabolic activation, induced a

highly significant dose-dependent mutagenic response (maximum increase was approximately 45 times the control mean response) at the *tk* locus in mouse lymphoma L5178Y cells in replicate trials. Furan was not tested for mutagenicity in the presence of metabolic activation.

5.3.2 DNA Damage

Wilson et al. (1992) reported that furan at doses up to 10,000 μM did not induce unscheduled DNA synthesis (UDS) in primary hepatocytes obtained from male $B6C3F_1$/CrlBR mice or male F344/CrlBR rats. NTP (1993; cited by IARC, 1995) conducted a study which concluded that furan, tested at 1.6 to 160 μg/mL (24 to 2350 μM) without S9 and 16 to 500 μg/mL (240 to 7350 μM) with S9, induced a significant increase in the frequency of SCE in CHO cells. The reproducible LED for the two trials without S9 was 160 μg/mL (2350 μM). Furan was weakly positive for the single trial with S9 with an LED of 500 μg/mL (7350 μM).

5.3.3 Chromosomal Damage

In a study conducted by Stich et al. (1981; cited by IARC, 1995), furan was positive for the induction of chromosome aberrations in CHO cells in the presence of S9 [LED = 4800 μg/mL (70,516 μM)] but negative in the absence of metabolic activation [HID = 220,000 μM (extrapolated from graph)]. Using a more sensitive protocol, NTP (1993) conducted a study which concluded that furan, tested at 100 to 500 μg/mL (1470 to 7350 μM) without S9 and 160 to 1000 μg/mL (2350 to 14,690 μM) with S9, was highly positive for clastogenicity in both the presence and absence of metabolic activation [LED = 100 μg/mL (1470 μM) for -S9 and 500 μg/mL (7350 μM) for +S9].

5.4 Mammalian Systems *In Vivo*

5.4.1 DNA Damage

Wilson et al. (1992) reported that furan at single gavage doses up to 200 mg/kg [2.93 mmol/kg] in male $B6C3F_1$/CrlBR mice or 100 mg/kg [1.47 mmol/kg] in male F344/CrlBR rats did not induce UDS in hepatocytes sampled at either 2 or 12 hours post treatment. In addition, NTP (1993) reported that furan at doses from 25 to 350 mg/kg (0.37 to 5.14 mmol/kg) administered i.p. did not induce SCE in bone marrow cells of male $B6C3F_1$ mice.

5.4.2 Chromosomal Damage

NTP (1993) concluded that furan was clastogenic in bone marrow cells sampled from male $B6C3F_1$ mice 36 hours (but not 17 hours) after a single i.p. treatment [LED = 250 mg/kg (3.67 mmol/kg)].

5.5 Genotoxicity of Structural Analogues

Studies of the genotoxic effects of furan structural analogues are summarized in Table 5-2

5.5.1 Chromosomal Aberrations

Sujatha et al. (1993) reported that 2-furyl methyl ketone (2-acetylfuran), a dietary furan, at single or multiple (daily for 5 days) doses up to 3000 ppm (0.044 μmol/kg) administered by gavage induced a weak clastogenic response in bone marrow cells of male Swiss albino mice sampled at 18-36 hours post treatment. However, the same treatment protocol was negative for the induction of chromosomal aberrations in spermatocytes in mice sampled from one to 35 days after treatment.

5.5.2 Sperm Morphology

Sujatha et al. (1993) also reported that 2-furyl methyl ketone (2-acetylfuran), at single or multiple (daily for 5 days) doses up to 3000 ppm (0.044 μmol/kg) administered by gavage was negative for the induction of sperm head abnormalities in mice sampled from one to 35 days after treatment.

TABLE 5-1. Summary of Furan Genotoxicity Studies[a]

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses	Endpoint Response	Comments	Reference
5.1 Noneukaryote Systems							
Salmonella typhimurium strains TA5135, TA1537, TA98, and TA100	*his* gene mutations, pre-incubation method	+/- rat and hamster S9	n.p.	33-3333 μg/plate (0.49 -49 μmol/plate)	negative/ negative	HID = 3333 μg/plate (49 μmol/plate)	Mortelmans et al. (1986)
S. typhimurium strains TA98 and TA100	*his* gene mutations, plate incorporation method	+/-	n.p.	0.8 - 100 μmol/plate	positive/ positive	A doubling in mutagenic response was present in TA100 only (+S9 LED = 20 μmol/plate; -S9 LED = 100 μmol/plate). Negative in TA98.	Lee et al. (1994)
Bacteriophage T7	Mutational inactivation, measured as growth in *Escherichia coli* B	-	n.p.	1-10 mg/mL (15,000-150,000 μM)	positive	Furan gave a mutagenicity index of 6.8, where values smaller than 8.0 are considered positive.	Ronto et al. (1992)
5.2 Lower Eukaryote Systems							
Drosophila melanogaster strain Canton-S males, mated to *Basc* females	Sex-linked recessive lethal mutations	NA	n.p.	10,000 ppm (146,908 μmol/kg) by feeding and 25,000 ppm (367,269 μmol/kg) by injection	negative	None	Foureman et al. (1994; cited by IARC, 1995)
5.3 Mammalian Systems *In Vitro*							
5.3.1 Gene Mutations							
L5178Y Mouse Lymphoma cells	*tk* gene mutations	-	n.p.	125 - 3800 μg/mL (1800-56,000 μM)	positive	dose response with maximum increase of approximately 45 times the control mean response, LED = 1139 μg/mL (17,000 μM)	McGregor et al. (1988; cited by IARC, 1995)
5.3.2 DNA Damage							
Primary male rat (F344/CrlBr) hepatocytes	Unscheduled DNA synthesis (UDS)	-	n.p.	Up to 10,000 μM	negative	study data not provided	Wilson et al. (1992)

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TABLE 5-1. Summary of Furan Genotoxicity Studies (Continued)[a]

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses	Endpoint Response	Comments	Reference
Primary male mouse (B6C3F$_1$/CrlBr) hepatocytes	UDS	-	n.p.	Up to 10,000 μM	negative	study data not provided	Wilson et al. (1992)
Chinese Hamster Ovary (CHO) cells	Sister chromatid exchanges (SCE)	+/-	n.p.	+S9: 16-500 μg/mL (240-7350 μM); - S9: 1.6-160.0 μg/mL (24-2350 μM)	positive/ positive	+S9; Considered weak positive response with LED = 500 μg/mL; -S9 LED = 160 μg/mL for replicate experiment	NTP (1993; cited by IARC, 1995)
5.3.3 Chromosomal Damage							
CHO cells	Chromosomal aberrations	+/-	n.p.	n.g.	positive/ negative	+S9 LED = 4800 μg/mL (70516 μM); -S9 HID = 220,000 μM (extrapolated from graph)	Stich et al. (1981; cited by IARC, 1995)
CHO cells	Chromosomal aberrations	+/-	n.p.	+S9: 160-1000 μg/mL (2350-14690 μM): -S9: 100-500 μg/mL (1470-7350 μM)	positive/ positive	+S9 LED = 500 μg/mL (7350 μM); - S9 LED = 100 μg/mL (1470 μM)	NTP (1993; cited by IARC; 1995)
5.4 Mammalian Systems *In Vivo*							
5.4.1 DNA Damage							
F344/CrlBr male rat	UDS in hepatocytes	NA	n.p.	5-100 mg/kg (0.07-1.47 mmol/kg)	negative	Sampled at 2 and 12 hours after treatment by gavage	Wilson et al. (1992)
B6C3F$_1$/CrlBr male mice	UDS in hepatocytes	NA	n.p.	10-200 mg/kg (0.15-2.93 mmol/kg)	negative	Sampled at 2 and 12 hours after treatment by gavage	Wilson et al. (1992)
B6C3F$_1$ male mice	SCE in bone marrow	NA	n.p.	25-350 mg/kg (0.37-5.14 mmol/kg)	negative	HID = 350 mg/kg (5.14 mmol/kg) i.p. after a single i.p. treatment	NTP (1993; cited by IARC, 1995)
5.4.2 Chromosomal Aberrations							
B6C3F$_1$ male mice	Chromosomal aberrations in bone marrow	NA	n.p.	87.5-350 mg/kg (1.29-5.14 mmol/kg) at 17 hr; 62.5-250 mg/kg (0.918-3.67 mmol/kg) at 36 hr	positive	Induction at 36 hr (but not 17 hr) after a single i.p. treatment; the LED = 250 mg/kg (3.67 mmol/kg).	NTP (1993; cited by IARC, 1995)

Table 5-2. Summary of Genotoxicity Studies of Furan Structural Analogue 2-Furyl Methyl Ketone

System	Biol. Endpoint	S9 Metab. Activation.	Purity	Doses	Endpoint Response	Comments	Reference
5.5 Mammalian Systems *In Vivo*							
5.5.1 Chromosomal Aberrations							
Male Swiss albino mice	Chromosome aberrations in bone marrow cells	NA	n.p.	1000-3000 ppm (0.015 - 0.044 μmol/kg) orally, single dose or daily doses for 5 days	positive	Induced a weak clastogenic response in bone marrow cells at 18-36 h primarily at the top dose; LED = 3000 ppm	Sujatha et al. (1993)
Male Swiss albino mice	Chromosome aberrations in primary spermatocytes	NA	n.p.	1000-3000 ppm (0.015 - 0.044 μmol/kg) orally, single dose or daily doses for 5 days	negative	Animals sampled from one to 35 days after treatment. HID = 3000 ppm	Sujatha et al. (1993)
5.5.2 Sperm Morphology							
Male Swiss albino mice	sperm head morphology	NA	n.p.	1000-3000 ppm (0.015 - 0.044 μmol/kg) orally, single dose or daily doses for 5 days	negative	Animals sampled from one to 35 days after treatment. HID = 3000 ppm	Sujatha et al. (1993)

[a] This table could include studies on furfural, tetrahydrofuran, *o*-anisidine, other closely related structural analogues, and on the ring-opened metabolite O:CHCH:CHCH:O.

Abbreviations: HID = highest ineffective dose; LED= lowest effective dose; n.p. = not provided; n.g. = not given; NA = not applicable

Figure 5-1. Genetic Activity Profile of Furan
(Data limited to IARC, 1995)
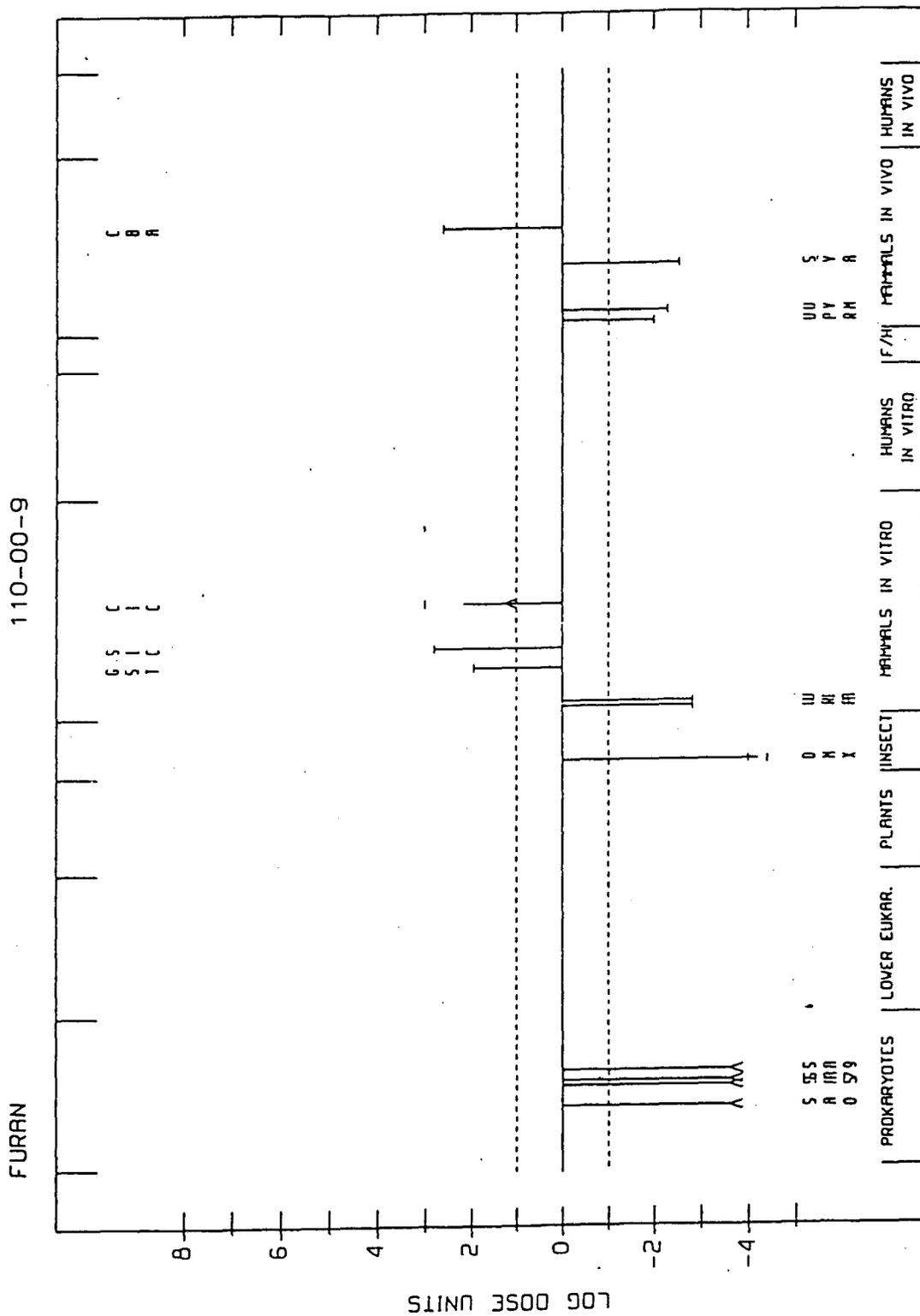
Figure 5-2. Schematic View of a Genetic Activity Profile
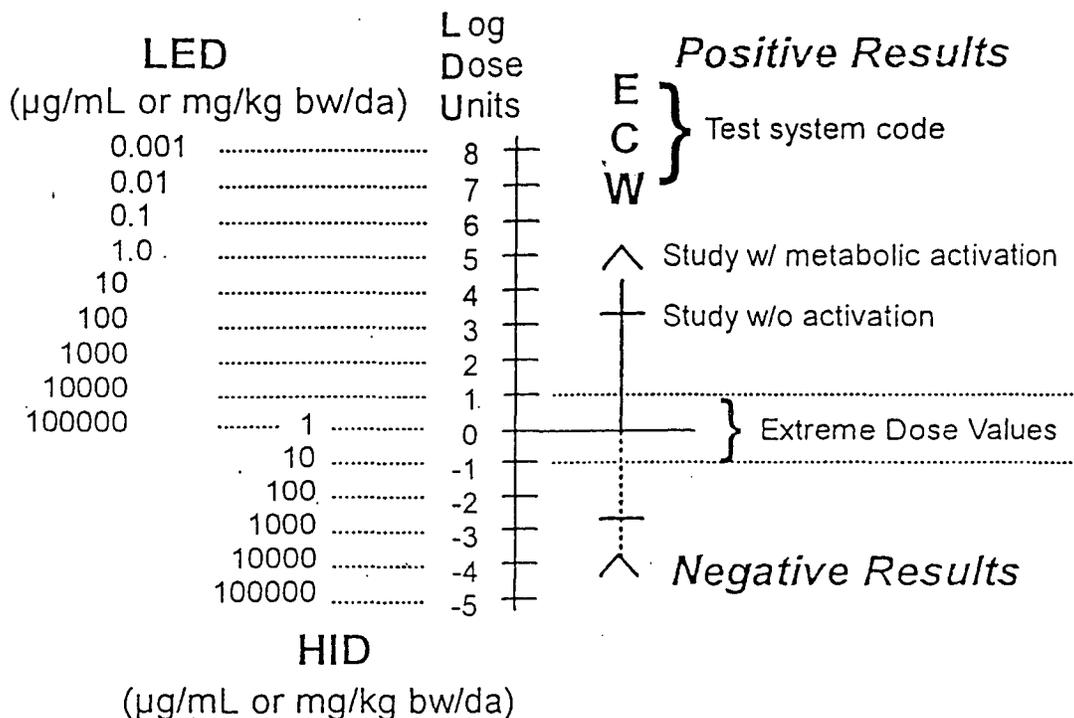
Figure 2. A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Waters MD, Stack HF, Garrett NE, Jackson MA (1991) The genetic activity profile database. Environmental Health Perspectives 96:41-45.

Waters MD, Stack HF, Brady AL, Lohman PHM, Haroun L, Vainio H (1988) Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutation Res 205:295-312.

Garrett NE, Stack HF, Gross MR, Waters MD (1984) An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutation Res 134:89-111.

6.0 OTHER RELEVANT DATA
6.1 Absorption, Distribution, Metabolism, Excretion

Summary: Respiratory tract retention of furan by mongrel dogs was shown to be directly related to the concentration of inhaled furan vapors. In the upper and lower respiratory tracts, there was also an inverse relationship between furan uptake and ventilatory rate of the dogs. When administered to male F344 rats by gavage, furan was retained mainly in the liver 24 hours after a single dosing. About 20% of the administered dose was eliminated in the urine and 22% was eliminated in the feces. Nineteen percent of the dose remained in tissues. Upon repeated dosing of male F344 rats, furan was retained mainly in the liver, less in the kidneys, and even less in blood. Once in the liver, furan became associated with liver protein, but not with liver DNA.

Only a few metabolites of furan have been identified. In male F344 rats treated by gavage, 14% of the original dose was expired as unchanged furan and 26% was expired as CO_2. Intermediates and other metabolites were not identified. In an *in vitro* system, *cis*-2-butene-1,4-dial-bissemicarbazone was reported to form in incubations of F344 rat liver microsomal preparations and furan.

6.1.1 Absorption

Egle and Gochberg (1979) evaluated the total respiratory tract uptake of inhaled furan by male and female mongrel dogs. Dogs (number not given) were anesthetized with pentobarbital sodium and attached to a respirometer that contained furan. They were then allowed to breathe spontaneously from the respirometer for 1 to 2 minutes. Their exhalations, as well as air samples from the respirometer, were collected and then analyzed for furan with a Beckman 72-5 Gas Chromatograph. A Chromosorb W column was used with helium as the carrier gas and a column temperature of 98 °C. It was determined that all animals inhaled 0.4-0.6 μg furan/mL (5.9-8.8 nmol/mL). The range of total respiratory uptake of furan was between 90.8 and 95.3%. Although it appeared that there was an inverse relationship between furan uptake and ventilatory rate (inhalations/min), statistical analysis revealed this to be insignificant.

When the effect of inhaled furan concentration was evaluated, it was found that as the concentration of the inhaled vapors increased from 0.21 to 0.55 μg/mL (3.1 to 8.1 nmol/mL), the percentage retained by the total respiratory tract increased. However, when the effect of tidal volume was evaluated, it was shown to have no significant effect on furan retention by the total respiratory tract.

While several studies were performed on the retention of furan by the total respiratory tract, the retention of furan in the lower respiratory tract was also evaluated in male and female mongrel dogs by Egle and Gochberg (1979). Each dog was fitted with an endotracheal tube and was allowed to breath spontaneously from the respirometer. Dogs inhaled 0.4-0.6 μg furan/mL (5.9-8.8 nmol/mL) and were found to have retained from 87.3 to 93.2% of this dose in the lower respiratory tract. There was a significant (p < 0.05) inverse relationship between furan uptake and ventilatory rate. The lowest average uptake (87.3%) occurred with the highest average ventilatory rate (24 inhalations/min) while the highest average uptake (93.2%) occurred with the lowest average ventilatory rate (7 inhalations/min).

Upper respiratory tract retention of furan by mongrel dogs was also found to have a significant (p-value not given) inverse relationship with ventilatory rate (Egle and Gochberg, 1979). This was found to be true in studies of both one-way and two-way upper respiratory retention. In these studies, dogs did not breathe spontaneously as they did in studies of total and lower respiratory tract retention. Instead, their tracheas were severed above the bifurcation at the area of the carina and they were exposed to furan vapors at predetermined tidal volumes (not specified) and rates (6-18 inhalations/min) through a mask which was placed proximal to the tracheal opening. For the one-way upper respiratory tract retention study, furan vapors were passed down the trachea and were collected at the distal end. For the two-way study, vapors were passed down the trachea into a 2-L syringe, which was placed at the distal end of the trachea. Furan vapors were drawn into the syringe and then returned upward and expelled through the nose, where they were collected for analysis. Average two-way upper respiratory tract retention of furan ranged from 85.4% (with an average of 18 inhalations/min) to 89.9% (with an average of 6 inhalations/min). Average one-way upper respiratory tract retention of furan was the same as two-way retention at all inhalation rates.

6.1.2 Distribution

The nature of furan distribution was assessed by Burka et al. (1991). Results from this study indicate that the pattern of furan distribution is related to its pattern of carcinogenicity. [^{14}C]Furan (8 mg/kg bw [117 µmol/kg bw], >99% pure) was administered to male F344 rats by gavage in corn oil either as a single dose or daily for 2, 4, or 8 days. Twenty-four hours after receiving a single dose, rats were found to have retained 19% of the administered radioactivity in tissue. Most of this radioactivity was found in the liver (13% of original dose). Fourteen other tissues or organs (including kidney, large and small intestines, glandular stomach, forestomach, lung, and blood) were assayed and were each found to have retained less than 0.5% of the original dose.

Upon repeated dosing, the concentration of radioactivity from labeled furan increased in liver, kidney, and blood. After 8 daily doses, most of the original dose was retained in liver (~1100 nmol eq/g tissue), less was retained in kidney (~325 nmol eq/g tissue), and even less was retained in blood (~35 nmol eq/g tissue). Furan retention in other tissues after multiple dosing was not evaluated (Burka et al., 1991).

The relatively high retention of furan in the liver corresponds to its selective carcinogenicity (NTP, 1993) in this organ in experimental animals. Further evaluation of furan-derived radioactivity in liver revealed it to be associated with liver protein, but not with liver DNA (radioactivity levels in DNA did not exceed background levels) (Burka et al., 1991). This conclusion was reached based on the assumption that since only 20% of the radioactivity in liver was extracted with organic solvents (methanol and ethyl acetate), the rest of the radioactivity must have been bound to liver tissue macromolecules. Levels of the non-extractable radioactivity in liver were shown to increase linearly over time with administration of repeated doses of [^{14}C]furan.

After Burka et al. (1991) revealed the propensity of [^{14}C]furan to bind to liver protein *in vivo*, Parmar and Burka (1993) verified this phenomenon *in vitro*. Liver microsomes (2 mg protein) from male F344 rats were incubated at 37°C for 5 minutes with 2 mmol [^{14}C]furan

(>99% pure) in the presence and absence of 2 mmol NADPH. After termination of the incubation, precipitated protein was repeatedly rinsed with methanol and then digested with NaOH. Furan-derived radioactivity was found to be covalently bound to microsomes in the absence of NADPH, but the presence of NADPH enhanced this binding by an order of magnitude. To determine which functional groups were responsible for the binding of [^{14}C]furan to microsomes, incubations were performed as previously described, but compounds containing free amine groups (semicarbazide), containing blocked amine groups but a free thiol group (N-acetylcysteine), or containing both free amine and thiol groups (glutathione) were also included. The addition of glutathione was most effective in inhibiting furan binding to microsomes. Semicarbazide was also an effective inhibitor, but N-acetylcysteine was only effective in inhibiting binding when microsomes had been previously treated with imidazole (an inducer of P-450 2E1).

6.1.3 Metabolism

Furan metabolism in male F344 rats was investigated by Burka et al. (1991). Twenty-four hours after administration by gavage of a single dose in corn oil of 8 mg [^{14}C]furan/kg bw (117 µmol/kg bw), 14% of the dose was expired as unchanged furan, with most of this (11% of the original dose) released within the first hour. Twenty-six percent of the original dose was expired as $^{14}CO_2$, indicating that ☐furan ring opening followed by complete oxidation of at least one of the labeled carbons was a major part of the overall metabolism of furan.☐ In addition, since radioactivity from labeled furan was shown to be associated with protein, especially liver protein (see section 6.1.2), it was proposed that furan was metabolized in the liver to one or more reactive intermediates, which were not identified in this report.

To assess the presence of metabolites in urine, samples were collected 24 hours after the administration of a single dose of 8 mg [^{14}C]furan/kg bw (117 µmol/kg bw), acidified with 10 µL acetic acid/mL and centrifuged at low g. HPLC analysis indicated that furan had been extensively metabolized. Ten peaks were observed, several of which were poorly resolved and were not identified. Further analysis with NMR did not aid in the identification of the isolates (Burka et al., 1991).

More recently, Chen et al. (1995) did identify an intermediate formed during furan metabolism. Furan in ethanol (0.025, 0.05, 0.1, 0.2, 2, and 20 mM) was incubated for 30 minutes at 37°C with F344 rat liver microsomal preparations in the potassium buffer (pH 6.8) in the presence of 25 mM glucose 6-phosphate, 2 units glucose-6-phosphate dehydrogenase/mL, 4 mM NADP$^+$, 3 mM MgCl$_2$, 1 mM EDTA, and 60 mM semicarbazide hydrochloride.

Incubations carried out in the absence of NADPH or microsomes served as controls. Metabolites were identified and quantified with HPLC using synthetically prepared standards of trans-2-butene-1,4-dial bissemicarbazone and cis-2-butene-1,4-dialbissemicarbazone.

Only trace amounts of a compound that coeluted with trans-2-butene-1,4-dial bissemicarbazone were detected in the incubations. The authors stated that this indicated that "little if any isomerization of the aldehyde had occurred". The formation of a compound that coeluted with cis-2-butene-1,4-dial-bissemicarbazone, however, was significant. The formation of this metabolite increased with increasing doses of furan but required NADPH and microsomes. Spectra generated from ^{1}H NMR, UV, and MS analyses indicated that the

metabolite was identical to the prepared standard of cis-2-butene-1,4-dial-bis-semicarbazone. The authors stated that this indicated that furan was oxidized by rat liver microsomes to cis-2-butene-1,4-dial. They also suggested that this metabolite "is probably responsible for the reactivity of furan with proteins in biological systems and is also likely to react with DNA".

No studies were found that evaluated the metabolism of inhaled furan.

6.1.4 Excretion

As mentioned in section 6.1.3, 24 hours after administration by gavage of a single dose of [^{14}C]furan (8 mg/kg; 117 μmol/kg) in corn oil to male F344 rats, 40% of the dose was eliminated unchanged or as $^{14}CO_2$ in exhaled breath. About 20% of the administered dose was eliminated in the urine and 22% was eliminated in the feces. Nineteen percent of the dose remained in tissues (Burka et al., 1991).

No studies were found that evaluated the excretion of inhaled furan.

6.2 Pharmacokinetics

Summary: Kinetic parameters, V_{max} (27.0 μmol/h furan) and K_M (2.0 μM furan), for furan biotransformation were determined for a 250-g rat. Furan biotransformation is a high affinity process as determined from the optimization of the fit of the PBPK model to the kinetic data by SimuSolv. Pharmacokinetic studies were conducted to validate the furan PBPK model using previously determined gas uptake and tissue partition coefficient data and blood and liver furan concentrations following constant inhalation exposure. These data suggest that the PBPK model developed for furan adequately described the pharmacokinetics of furan in rats exposed to constant inhalation concentrations. Xenobiotic biotransformation kinetics determined in freshly isolated hepatocytes quantitatively reflect in vivo pharmacokinetics for furan, which is metabolized primarily by the liver.

The gas uptake kinetics of furan were well described by a single saturable process following Michaelis-Menten kinetics after Charles River F-344 rats (N=3 males per dose group) were exposed to 100, 500, 1050, or 3850 ppm furan (>99% pure) in the air of separate recirculating 9.1-L glass chambers. The atmosphere was serially sampled via an automatic gas sampling valve every 10 min for 2.7 h (100 ppm), 3.5 h (500 ppm), 4.7 h (1000 ppm), and 5.8 h (1500 ppm) and then analyzed by gas chromatography (GC) equipped with a flame ionization detector. In these studies and subsequent experiments mentioned below, the physiological parameters used to describe the pharmacokinetics in F-344 rats during inhalation and oral exposures were estimated independently and held fixed (Arms and Travis, 1988, body weight, flow rate [cardiac output and alveolar ventilation]); measured in independent experiments (Gargas et al., 1989, partition coefficients determined in vitro using vial equilibration); or estimated by fitting the model to the data (Burka et al., 1991, oral absorption rate; Kedderis et al., 1993, metabolic constants, V_{maxc} and K_M). The basic structure of the PBPK model was a modification of the PBPK model of Ramsey and Andersen (1984; cited by Kedderis et al., 1993), with the mass balance equations used those previously described by Gargas et al. (1986; cited by Kedderis et al., 1993) and all furan biotransformation assumed to take place in the liver and to follow Michaelis-Menten kinetics. Furan concentrations in the chamber atmosphere during gas

uptake experiments were determined from standard curves generated with furan vapors collected in 10-L Tedlar gas sampling bags. Kedderis et al. (1993) found that furan biotransformation is a high affinity process as determined from the optimization of the fit of the PBPK model to the kinetic data by SimuSolv (Dow Chemical Co., curve-fitting software). Kinetic parameters, V_{max} (27.0 μmol/h furan) and K_M (2.0 μM furan), for furan biotransformation were determined for a 250-g rat. In some experiments, rats were pretreated with pyrazole (320 mg/kg i.p.) 30 min prior to furan exposure. Although the data for these experiments were not shown, the authors stated that pretreatment with pyrazole completely inhibited furan biotransformation. These findings are consistent with oxidative metabolism of furan by cytochrome P-450 (Kedderis et al., 1993), whose activity is inhibited by pyrazole.

Subsequent pharmacokinetic studies were conducted to validate the furan PBPK model developed from gas uptake experiments and tissue partition coefficients (measured: blood:air, 6.59; liver:air, 5.94; fat:air, 64.06; rapidly perfused:air, 5.94; slowly perfused:air, 4.23; fitted:V_{maxc}, 71.4 μmol/kg/h). Fischer-344 rats (N=12 per dose group) were exposed to 52, 107, or 208 ppm furan (constant inhalation concentrations) for up to 4 h in a battery jar chamber, and furan concentrations in blood and liver were determined at various time points (4.0 to 4.8 h) following exposure. Three rats per time point were anesthetized with CO_2 and blood was removed from the inferior vena cava using a heparinized gas-tight syringe. One milliliter aliquots of blood were immediately transferred to chilled vials sealed with Teflon-lined septa. A 1 mL aliquot of the headspace was analyzed for furan by GC after blood samples were incubated with shaking for 1 h at 37°C. Control experiments (data not shown) showed that equilibrium between furan in blood and headspace was reached within 1 h under these conditions. The concentration of furan in blood was determined from standard curves prepared in blood under the conditions described above. The liver of each rat was excised and a 4-g section transferred to a 20-mL vial containing succinic acid buffer and briefly homogenized following blood sampling. The vials were then sealed immediately and incubated with shaking at 37°C for 1 h. Control experiments (data not provided) showed that the unfavorable pH of succinic acid buffer prevented further metabolism under these conditions. Within 1 h, equilibrium between liver homogenate and the headspace was achieved. Gas chromatography and GC/MS were used to determine furan in the vial headspace following incubation. The measured blood and liver concentrations of furan following inhalation exposure were relatively close to the predictions of the furan PBPK model. SimuSolv optimization of V_{maxc} and K_M using the data generated in the 4-h gas inhalation study yielded a V_{maxc} of 69.2 μmol/h/kg and a K_M of 1.7 μM. The differences between the model simulations and the experimentally determined tissue concentrations are probably due to loss of volatile furan during transfer of the tissues (Kedderis et al., 1993). The rate of furan loss from aqueous media at 37° C was 0.153 min^{-1} (Wilson et al., 1992; cited by Kedderis et al., 1993). In a nonmetabolizing tissue exposed to the open air, this rate predicts a half-life of 4.5 min for furan. The kinetic parameter values determined using data generated from rats exposed to constant inhalation concentrations of furan are similar to those obtained after optimization of the fit of the PBPK model to the kinetic data by SimuSolv. These data suggest that the PBPK model developed for furan adequately described the pharmacokinetics of furan in rats exposed to constant inhalation concentrations.

Model simulations of furan metabolism 24 h after an oral dose of 8 mg/kg bw (117 μmol/kg bw) conducted by Kedderis et al. (1993) were in agreement with the experimentally derived data of Burka et al., 1991. Burka et al. found that 24 h after dosing rats p.o. with 8 mg/kg bw (117 μmol/kg bw) furan, 14% of the dose was exhaled unchanged and 86% was metabolized. These data are in agreement with model simulations using a k_A (oral absorption rate; estimated from the disposition data of Burka et al. [1991]) of 2 h^{-1}, which predicted 86% of the dose metabolized and 14% exhaled unchanged 24 h after administration of the dose.

The kinetics of furan biotransformation by freshly isolated F-344 rat hepatocytes (2 x 10^6 cells/mL in a 3-mL suspension) *in vitro* under an atmosphere of 5% CO_2 in air were investigated by Kedderis et al. (1993). Furan biotransformation by viable hepatocytes (>80% as determined by trypan blue exclusion) was described as a single-saturable process, with a V_{max} of 0.018 μmol/h/10^6 cells and a K_M of 0.4 μM. Based on the estimate of 128 x 10^6 hepatocytes/g liver (Seglen, 1976; cited by Kedderis et al., 1993), the V_{max} for furan oxidation by rat hepatocytes *in vitro* was extrapolated to the whole animal *in vivo*. A V_{maxc} of 60.8 μmol/h/kg and a K_M of 0.4 μM were obtained after conversion of the units of the extrapolated *in vitro* kinetic parameters to those used in the furan PBPK model. When used in the PBPK model, the extrapolated kinetic parameters for furan oxidation accurately predicted the *in vivo* gas uptake data. These results indicated that xenobiotic biotransformation kinetics determined in freshly isolated hepatocytes quantitatively reflect *in vivo* pharmacokinetics for furan, which is metabolized primarily by the liver.

It should be noted that the V_{max} extrapolated from hepatocytes *in vitro* to the whole rat (23.0 μmol/h) was in close agreement with that determined from the gas uptake kinetic studies (27.0 μmol/h). However, the K_M determined *in vitro* (0.4 μM) was lower than that estimated *in vivo* (2.0 μM). The difference in K_M values is likely due to blood-flow limited delivery of furan to the liver *in vivo* (Kedderis et al., 1993).

Sensitivity analysis of the model parameters with respect to the gas uptake data revealed that only 4 parameters (alveolar ventilation rate, blood flow to the liver, blood:air partition coefficient, and V_{maxc}) caused a >1% change in data simulations when they were varied by 1% (data not shown). The changes in data simulation were concentration- and time-dependent and did not exceed 3%. The sensitivity to V_{maxc} and hepatic blood flow is consistent with rapid furan metabolism, which is blood-flow-limited at low concentrations. Since inhalation is the route of exposure and exhalation of furan is a significant excretion pathway (Burka et al., 1991), the sensitivity of the data simulations to alveolar ventilation rate and blood:air partition coefficient was expected (Kedderis et al., 1993). In fact, similar parameter sensitivities have been described for the gas uptake kinetics of dichloromethane (Burka et al., 1991).

6.3 Modes of Action

Summary: Furan-induced carcinogenesis is thought to proceed via metabolic activation of furan by P-450 to a reactive and cytotoxic intermediate that stimulates cell replication, increasing the likelihood of tumor induction. [Although furan is known to induce both cellular proliferation and tumorigenesis in the liver of experimental animals, it has not been shown unequivocally that

these two events are mechanistically related, although some do believe this to be the case.] The postulated reactive metabolite is cis-2-butene-1,4-dial. This reactive metabolite probably explains furan's binding reactivity with proteins both in vitro (uninduced and induced F344 male rat liver microsomes) and in vivo (F344 male rat liver protein) in biological systems.

The activation of oncogenes may also be at least partly responsible for the carcinogenicity of furan. Both spontaneously occurring and furan-induced tumors in B6C3F₁ mouse liver were reported to have a high frequency of activated H-ras and in some furan-induced, but not spontaneous, tumors activated K-ras was detected. In male F344 rats treated with a single dose of furan by gavage, myc and fos expression became elevated 1 to 2 hours following furan administration and remained elevated for as long as 2 days. In male and female F344 rats treated with furan by gavage for up to 6 weeks, myc expression became elevated in male rat liver beginning at week 3 and in female rat liver beginning at week 6. When male B6C3F₁ mice were similarly treated, however, myc expression did not become elevated. A c-met-encoded HGF/SF receptor protein, confirmed to be c-Met p 140 was detected in hepatic adenocarcinomas and transplants of these tumors derived from furan-treated rats.

6.3.1 The Role of Cytochrome P-450

The mechanism by which furan is carcinogenic in experimental animals has not been elucidated, but several studies (see Chen et al., 1995, for review) suggest that it may be mediated via cytochrome P-450. A current hypothesis for the molecular mechanism of furan-induced carcinogenesis is metabolic activation of furan by P-450 to a reactive and cytotoxic intermediate that stimulates cell replication, increasing the likelihood of tumor induction (Chen et al., 1995; Kedderis et al., 1993). The postulated reactive metabolite is cis-2-butene-1,4-dial, which was recently characterized as a furan metabolite by Chen et al. (1995). This reactive metabolite probably explains furan's binding reactivity with proteins both in vitro (uninduced and induced F344 male rat liver microsomes) and in vivo (F344 male rat liver protein) in biological systems (Burka et al., 1991; Parmar and Burka, 1993). Although reaction of furan metabolites with DNA is possible, Burka et al. (1991) found no binding of [¹⁴C]furan to the DNA isolated from livers of rats treated with [¹⁴C]furan.

Furan metabolism can be blocked by pyrazole, a cytochrome P-450 inhibitor. The binding of furan metabolite(s) to protein can be blocked by glutathione and semicarbazide. Biologically relevant concentrations of furan in freshly isolated hepatocytes reduce cell viability, and the ensuing oxidation of furan is accompanied by depleted glutathione levels. The cytochrome P-450 inhibitor 1-phenylimidizole inhibits these reactions and pretreatment with acetone, known to be a cytochrome P-450 2E1 inducer, enhances them in vitro (Carfagna et al., 1993; Kedderis et al., 1993). The P-450 inhibitor diethyl carbamate reduces the hepatotoxicity of furan in vivo (Masuda et al., 1984; cited by Carfagna et al., 1993). Thus, the effects of P-450 inducers and inhibitors on furan metabolism and binding in vitro and in vivo support the role for P-450 in the metabolism of furan to a reactive metabolite, which interacts irreversibly with a specific cellular target and is involved in cell death (Carfagna et al., 1993).

While the number of studies that evaluated the role of P-450 in the activation of furan is limited (see, for example, Carfagna et al., 1993; Parmar and Burka, 1993), there are a number of studies indicating that some structural analogues of furan are activated to reactive metabolites via

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P-450 metabolism. This has been demonstrated *in vitro* for furosemide (for reviews see Burka and Boyd, 1985; Boyd, 1982; McMurty and Mitchell, 1977), 4-ipomeanol, 3-methylfuran, furamide, and 2-(*N*-ethylcarbamoylhydroxymethyl)furan (for reviews see Burka and Boyd, 1985; Boyd, 1982).

6.3.2 Cellular Proliferation and Tumorigenesis

Although furan is known to induce both cellular proliferation (see Table 6-1) and tumorigenesis (see Table 4-1) in the liver of experimental animals, it has not been shown unequivocally that these two events are mechanistically related (for review see Huff et al., 1993), although some do believe this to be the case (e.g., Chen et al., 1995; Kedderis et al., 1993; Wilson, et al., 1992).

In contrast to Chen et al. (1995), Kedderis et al. (1993), and Wilson et al. (1992), Huff (1993) stated that he did "not believe...the notion that simply increasing cell turnover leads to or causes cancer...". Huff (1993) reached this conclusion by reviewing nearly 1800 sex-species control and exposure group experiments on the carcinogenicity of 130 chemicals (including furan). Within this review, the carcinogenicity of 53 chemicals, evaluated in 99 two-year studies, was assessed. It was found that only a small fraction of chemicals (7/53) which induced tumorigenesis in at least one organ in one sex of one species also induced toxicity at the site of tumorigenesis. The identity of these 7 chemicals was not revealed in the article.

While the review by Huff (1993) focused more on the overall relationships between chemically induced cytotoxicity, cellular proliferation, and tumorigenesis than on the identities of the evaluated chemicals, it did specifically address a 6-week study conducted by Wilson et al. (1992) on the effect of furan (administered by gavage) on cellular proliferation and carcinogenesis in F344 rats (administered 8 mg/kg/day [117 µmol/kg/day]) and B6C3F$_1$ mice (administered 15 mg/kg/day [220 µmol/kg/day]). Although Wilson et al. (1992) concluded that the cytotoxicity, and resulting cellular proliferation (see Table 6-1 for details), induced by furan "likely had a significant impact on tumor development," Huff (1993) did not agree with this because of an inconsistency he found in the results. He noted that while furan induced a higher level of cellular proliferation in female mice than in male mice, male mice had a greater carcinogenic response to furan. A hypothesis that accounts for the lower carcinogenic response in the females is that the females had a higher cellular apoptosis:cellular proliferation ratio than did the males. In a review by Goldsworthy et al. (1996) it was noted that "the regulation of cell death through apoptosis has a critical influence on multiple stages of hepatocarcinogenesis".

6.3.3 Activation of Oncogenes

The conversion, via genetic mutation, of proto-oncogenes to oncogenes is thought to be involved in the neoplastic transformation of cells (for reviews see Maronpot et al., 1995; Reynolds et al., 1987). Since several studies indicate that furan is capable of inducing such conversions in cells of experimental animals (see following review), the activation of oncogenes may be at least partly responsible for the carcinogenicity of furan. A study by Reynolds et al. (1987) provides support for this hypothesis. Spontaneously occurring and furan-induced B6C3F$_1$ mouse liver adenomas and carcinomas were examined for the presence of activated oncogenes. The B6C3F$_1$ mouse is known to have a high incidence of spontaneously occurring tumors with activated oncogenes (Reynolds, et al., 1986; for review see Maronpot et al., 1995) and the study

was undertaken to determine whether there was a quantitative and/or qualitative difference in oncogene activation between these tumors and furan-induced tumors.

Tumors in both control and furan-treated mice were found to have a high frequency of activated H-*ras*. Three of 10 adenomas in control mice, 12/17 carcinomas in control mice, 7/19 adenomas in furan-treated mice, and 3/10 carcinomas in furan-treated mice had activated H-*ras* genes. Further analysis revealed that while all of the activated H-*ras* genes in spontaneous tumors (adenomas and carcinomas) had point mutations at codon 61, only about 60% (4/7) of H-*ras* mutations in furan-induced adenomas and 30% (1/3) of H-*ras* mutations in furan-induced carcinomas did. Except for 1 activated H-*ras* gene in a furan-induced carcinoma, the other mutations in furan-induced tumors all occurred at codon 117. The location of the mutation in the remaining H-*ras* gene in the furan-induced carcinoma was not identified, although it was determined that it was not at codon 13.

In addition to activated H-*ras* genes, both spontaneous (1/17) and furan-induced (1/10) carcinomas were found to have an activated *raf* gene and 2/19 furan-induced (but not spontaneous) adenomas had activated K-*ras*. The authors proposed 2 mechanisms that could have been responsible for the difference in the pattern of *ras* activation between spontaneous and furan-induced liver tumors. In the first proposed pathway, furan induced mutations in proto-oncogenes, but this induction occurred secondary to the cytotoxic effects of furan. The authors ruled this mechanism unlikely since no cytotoxic lesions were observed in the livers of mice administered carcinogenic doses of furan for 90 days. The other, more likely, mechanism for the activation of oncogenes by furan is that it acts as a direct genotoxin.

Although Reynolds et al. (1987) detected activated K-*ras* in some furan-induced liver tumors, Sirica (1996), using polymerase chain reaction analysis, did not detect mutated K-*ras* oncogenes in hepatic adenocarcinomas or tumor transplants from rats exposed *in vivo* to furan. Using immunohistochemical analysis and the Mutant p53 ImmunoCruz□ System, Sirica (1996) also did not detect mutated p53 in these tumors, but did detect a c-*met*-encoded HGF/SF receptor protein, confirmed to be c-Met p 140.

A later study by Butterworth et al. (1994) evaluated the expression of *myc*, *fos*, and Ha-*ras* in the livers of male F344 rats treated with a single dose of furan, and male and female F344 rats and male B6C3F₁ mice treated short-term with furan. In the acute study, male rats were administered furan in corn oil by gavage (30 mg/kg [441 μmol/kg]) and were killed 12 h, 1 day, 2 days, 4 days, or 8 days later. The expression of *myc* increased as early as 1 hour after treatment, peaked at about 12 hours after treatment, and remained elevated for as long as 2 days. Expression of *fos* became elevated 2 hours after treatment, peaked after 12 hours, and remained elevated for as long as 2 days. Ha-*ras* expression peaked 24 hours after furan treatment.

In the short-term studies done by Butterworth et al. (1994), male and female F344 rats were administered furan in corn oil by gavage (8 mg/kg/day [117 μmol/kg/day]) for up to 6 weeks and male B6C3F₁ mice were similarly administered 15 mg furan/kg (220 μmol/kg) for up to 6 weeks. There was a large increase in the expression of *myc* in male rat liver beginning at week 3 and increasing at week 6 up to 15-fold over control levels. In treated female rats, *myc* was shown to increase at week 6, but only 3-fold over control levels. There was no significant increase in *myc* expression in male mice liver, nor was there a significant increase in *fos*

expression in any animals (male and female rats and male mice). Ha-*ras* expression was significantly increased only in male rats, and only at week 3.

Sprankle et al. (1994) reported that c-*met* was not overexpressed in male F344 rats treated with a single dose of furan or in male B6C3F₁ mice and male and female F344 rats treated short-term with furan. Similar to the study by Butterworth et al. (1994), in the acute study by Sprankle et al. (1994), male rats were administered a single dose (30 mg/kg [441 mg/kg]) of furan in corn oil by gavage and killed 12 h, 1 day, 2 days, 4 days, or 8 days after treatment. Although the livers of these animals displayed regenerative cell proliferation following treatment, no increase in the expression of c-*met* was detected.

In the short-term studies done by Sprankle et al. (1994), male and female F344 rats were administered furan in corn oil by gavage (8 mg/kg/day [117 µmol/kg/day]) for up to 6 weeks and male B6C3F₁ mice were similarly administered 15 mg furan/kg (220 µmol/kg) for up to 6 weeks. Like animals in the acute study, these animals were found to have regenerative hyperplasia in the liver following treatment, but c-*met* was not overexpressed in this organ.

Crist et al. (1992 abstr.) investigated p53 protein expression in tumors from furan-treated rats. Cholangiocarcinomas from F344 rats treated with furan (dose not specified) orally 5 days/wk for 103 consecutive weeks were used in the analysis. Nuclear staining indicated that none of the tumors overexpressed a mutant p53 protein product.

6.4 Structure-Activity Relationships

The results of several studies indicate that furan and some of its analogues, including 2-methylfuran, 2-(*N*-ethylcarbamoylhydroxymethyl)furan, 3-methylfuran, 4-ipomeanol, furamide, furosemide, and ngaione require P-450-mediated metabolism for activation (see section 6.3.1 for review of furan activation by P-450, see Ravindranath et al., 1986; cited by Parmar and Burka, 1993, for study of 2-methylfuran activation, and see Burka and Boyd, 1985, for review of activation of other furan analogues). Other studies suggest that the furan ring of these compounds is the targeted functional group for P-450-mediated activation (McMurtry and Mitchell, 1977; for reviews see Burka and Boyd, 1985; Boyd, 1982).

Although the P-450-mediated activation of furan and some of its analogues produces reactive metabolites, the cellular damage induced by these compounds varies among analogues. For example, furan analogues vary in their clastogenicity in CHO cells (Stich et al., 1981), and in their genotoxicity in *Salmonella typhimurium* (Lee et al., 1994) and phage T7 (Ronto et al., 1992; Ronto et al., 1989). This difference in clastogenicity and/or mutagenicity may be explained by the type of substituent found on the furan ring. For example, electron-withdrawing substituents have been shown to render the furan ring more resistant to oxidation (for review see Burka and Boyd, 1985) and, presumably, to P-450-mediated activation.

6.5 Cell Proliferation

Full experimental details for the studies described in this section are presented in Table 6-1.

Summary: In brief, the hepatocyte labeling index was significantly increased in female B6C3F$_1$ mice (males not evaluated) administered furan by gavage for 4 weeks and in male B6C3F$_1$/CrlBR mice (females not evaluated) and male and female F344/CrlBR rats administered furan by gavage for 1, 3, or 6 weeks. Male B6C3F$_1$/CrlBR mice and male F344/CrlBR rats (females not evaluated) administered a single dose of furan by gavage displayed a peak in the hepatocyte labeling index 48 hours after treatment. The incidence of hyperplasia of the liver (detected via gross or microscopic examination) was significantly increased in male F344 rats (females not evaluated) administered furan by gavage for 7 days, in female, but not male, B6C3F$_1$ mice and in male and female F344/N rats administered furan by gavage for 13 weeks, and in male and female B6C3F$_1$ mice administered furan by gavage for 2 years.

6.5.1 Mice

Administration of 4, 8, or 15 mg furan (59, 117, or 220 μmol) per kilogram mean body weight per day, 5 consecutive days/wk for 3 weeks, by gavage to 50-day-old female B6C3F$_1$ mice did not significantly increase the hepatocyte labeling index as compared to solvent controls (experiment 1). However, administration of 15 mg/kg/day (220 μmol/kg/day) did significantly increase the hepatocyte labeling index when the dosing schedule was altered (3 consecutive days of dosing, followed by 2 weeks of 5 consecutive days, and a final week of 2 consecutive days; experiment 2). In addition, when controls from experiment 1 were pooled with controls from experiment 2, there was a significant increase in the hepatocyte labeling index in mice treated with 8 or 15 mg/kg (117 or 220 μmol/kg) in experiment 1 as compared to pooled vehicle controls (Fransson-Steen et al., 1996 [in press]).

In both experiments 1 and 2, the plasma activity of liver-related enzymes (alanine aminotransaminase and sorbital dehydrogenase) and the level of total bile acid were significantly increased (2- to 3-fold) in mice treated with 15 mg furan/kg (220 μmol/kg; data not shown). In addition, there was a dose-related increase in minor subcapsular inflammation with little or no necrosis in mice treated with 8 or 15 mg/kg (117 or 220 μmol/kg). Treatment with 15 mg furan/kg (220 μmol/kg) + aminobenzotriazole (ABT) completely prevented furan-induced inflammation and necrosis, as well as the elevation in plasma activity of liver enzymes and bile acid levels. However, mice treated with furan + ABT displayed hepatocyte vacuolization which was not detected in animals treated with furan alone or in vehicle controls (Fransson-Steen et al., 1996 [in press]).

Administration of a single dose of 50 mg (734 μmol) furan per kilogram mean body weight by gavage to 8- to 10-week-old male B6C3F$_1$/CrlBR mice caused a peak in the hepatocyte labeling index 48 hours after treatment (< 0.5 at 12 h, < 0.5 at 24 h, 23.9 at 48 h, ~2.0 at 4 days, and < 0.5 at 8 days vs. < 0.5 in vehicle controls at 48 h) (Wilson et al., 1992; Butterworth et al., 1994; Sprankle et al., 1994; IARC, 1995). The timing for the peak appearance of labeling is consistent with the expectation that the proliferative response arose as repair activity following tissue damage rather than as a result of mitogenic activity.

When repeated doses of furan (15 mg/kg/day [220 μmol/kg/day], 5 days/wk for 1, 3, or 6 weeks) were administered by gavage to 10- to 12-week-old male B6C3F$_1$/CrlBR mice, there was an increase in the hepatocyte labeling index at all measured time points during continual dosing (25.1 at wk 1, 12.0 at wk 2, and 3.2 at wk 6 vs. control values of 0.41 at wk 1 and 0.89 at wk 6). Males and females treated for 6 wk had bile-duct hyperplasia and metaplasia, resembling intestinal cells, in areas of liver fibrosis (Wilson et al., 1992; Butterworth et al., 1994; Sprankle et al., 1994; IARC, 1995).

Oral furan administration for 13 weeks to 51-day-old male (2, 4, 8, 15, or 30 mg/kg/day [29-441 μmol/kg/day]) and female (4, 8, 15, 30, or 60 mg/kg/day [59-881 μmol/kg/day]) B6C3F$_1$ mice significantly increased the incidences of biliary tract hyperplasia and cholangiofibrosis in females, but not males, treated with 30 or 60 mg [41 or 881 μmol] furan per kilogram mean body weight. The incidence of hepatocyte cytomegaly was also significantly increased in males treated with 30 mg [441 μmol] furan and in females treated with 30 or 60 mg [441 or 881 μmol] furan (NTP, 1993).

In a 2-year study conducted by the NTP (1993), oral administration of furan (8 or 15 mg/kg/day [117 or 220 μmol/kg/day]) to 58-day-old male and female B6C3F$_1$ mice significantly increased the incidences of focal hyperplasia of the liver, biliary tract chronic inflammation, biliary tract fibrosis, biliary tract hyperplasia, and hepatocyte cytomegaly in males and females treated with either dose.

6.5.2 Rats

Administration of a single dose of 30 (441 μmol) furan per kilogram mean body weight by gavage to 8- to 10-week-old male F344/CrlBR rats caused a peak in the hepatocyte labeling index 48 hours after treatment (< 0.5 at 12 h, < 0.5 at 24 h, 17.8 at 48 h, ~1.5 at 4 days, and < 0.5 at 8 days vs. < 0.5 in vehicle controls at 48 h) (Wilson et al., 1992; IARC, 1995).

When repeated doses of furan (8 mg/kg/day [117 μmol/kg/day], 5 days/wk for 1, 3, or 6 weeks) were administered by gavage to 10- to 12-week-old male and female rats, there was an increase in the hepatocyte labeling index at all measured time points (males: 3.2 at wk 1, 9.2 at wk 3, and 6.5 at wk 6; females: 11.7 at wk 1, 9.2 at wk 3, and 14.4 at wk 6; controls: 0.08 at wk 1 and 0.29 at wk 6 [males], 0.77 at wk 1 and 0.75 at wk 6 [females]) (Wilson et al., 1992; Butterworth et al., 1994; Sprankle et al., 1994; IARC, 1995).

In a short-term study conducted by the NTP (1993), oral administration of furan for 12 days to 47-day-old male (5, 10, 20, 40, or 80 mg/kg/day [73-1175 μmol/kg/day]) and female (10, 20, 40, 80, or 160 mg/kg/day [147-2350 μmol/kg/day) F344/N rats produced mottled and enlarged livers in males treated with 20, 40, or 80 mg/kg/day (294-1175 μmol/kg/day) and in females treated with 40, 80, or 160 mg/kg/day (588-2350 μmol/kg/day).

In young adult male F344 rats, treatment with 60 mg (881 μmol) furan per kilogram mean body weight per day by gavage for 10 to 14 days produced atrophy of the right liver lobe. This was associated with the appearance of cholangiolar-like structures composed of biliary epithelial cells and usually a single ductular hepatocytic cell in various stages of maturation. Unlike normal cholangioles, the nuclear dimensions and phenotypic features of these structures were consistent with a differentiation of rare bile ductular-like cells to transitional ductular cells

to more mature ductular hepatocytes (Sirica et al., 1994b).

Treatment of young adult male F344 rats with 15, 30, 45, or 60 mg (220-881 μmol/kg/day) furan per kilogram mean body weight per day by gavage for 3 weeks induced the rapid development of cholangiofibrosis (localized to the caudate lobe) in rats treated with 45 or 60 mg/kg/day (661 or 881 μmol/kg/day). The caudate lobe was characterized by the presence of well-differentiated hyperplastic bile ductules and of metaplasia resembling metaplastic intestinal glands supported by a fibrous connective tissue stroma. Other liver lobes (i.e., median and left lateral) were characterized mainly by cirrhosis and areas of hepatocellular degeneration and necrosis (detected sporadically within Zones 2 and 3 of the liver acini) (Elmore and Sirica, 1991). When rats of the same sex and age were treated with 45 mg (661 μmol) furan per kilogram mean body weight per day by gavage for 1-32 days, prominent bile ductule hyperplasia was observed on day 7 in tissue sections of the right liver lobe (and to a lesser extent in the caudate lobe) exhibiting marked loss of normal liver parenchyma. In addition, on days 9 to 32 there was a sharp weight loss in the right liver lobe that corresponded to a sharp rise in the incidence of cholangiofibrosis. By day 32, much of the right lobe had been replaced with cholangiofibrotic tissue. This effect was much less pronounced in the caudate liver lobe (Elmore and Sirica, 1992).

In another experiment using young adult male F344 rats, treatment with 45 mg (661 μmol) furan per kilogram mean body weight per day (following bile duct ligation one week before) by gavage for 5 to 6 weeks resulted in the replacement of 72.6 ± 16.3% of the liver with well-differentiated hyperplastic bile ductules and greatly increased activities of γ-glutamyl transpeptidase in liver homogenates. The livers of ligated vehicle controls had 20.0 ± 4.2% bile-duct tissue and the livers of rats receiving furan treatment after a sham operation had 11.9 ± 3.1% bile-duct tissue (Sirica et al., 1994a).

Treatment of 51-day-old male and female F344/N rats with 4, 8, 15, 30, or 60 mg (59-881 μmol) furan per kilogram mean body weight per day by gavage for 13 weeks significantly increased the incidence of biliary tract hyperplasia in males and females treated with any of the doses as compared to vehicle controls (see Table 6-1 for incidences) (NTP, 1993).

In a stop-exposure study (Maronpot et al., 1991; NTP, 1993), treatment of 6-week-old male F344 rats with 30 mg (441 μmol) furan per kilogram mean body weight per day by gavage for 13 weeks produced enlarged livers with areas of fibrosis and cellular hyperplasia in animals killed at 90 days. Rats killed after 9 and 15 months had similar (in terms of severity and extent) liver abnormalities.

Table 6-1. Cell Proliferation Induced by Furan

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice							
50-day-old B6C3F₁ mouse	6-11F (for each dose)	6-11F (corn oil alone)	furan, purity not specified	4, 8, or 15 mg/kg/day (59, 117, or 220 μmol/kg/day) by gavage	5 consecutive days/wk for 3 wk	Animals were necropsied on the day following the last treatment. Seven days prior to necropsy, mini-osmotic pumps containing 30 mg BrdU/mL were surgically implanted. Liver sections were immunohistochemically stained for BrdU and labeling indices (percent labeled hepatocyte nuclei/~2000 non-labeled hepatocyte nuclei) were obtained from random fields of the median lobe. In both the 3- and 4-week studies, the plasma activity of liver-related enzymes (alanine aminotransaminase and sorbital dehydrogenase) and the level of total bile acid were significantly increased (2- to 3-fold) in animals treated with 15 mg furan/kg (220 μmol/kg; data not shown). In addition, there was a dose-related increase in minor subcapsular inflammation with little or no necrosis in animals treated with 8 or 15 mg/kg (117 or 220 μmol/kg).	Fransson-Steen et al. (1996, in press)
	6-11F (furan) 6-11F (furan + i.p.- administered aminobenzotriazole [ABT; 100 mg/kg, 7 days/wk, beginning 5 days before start of furan dosing]) (ABT is a P-450 inhibitor)			15 mg/kg/day (220 μmol/kg/day) by gavage	3 consecutive days followed by 2 wk of 5 consecutive days and a final wk of 2 consecutive days	*first experiment*: Liver: Negative Liver weight was not affected by furan treatment. Although there was a slight dose-dependent increase in the hepatocyte labeling index in animals treated with 4, 8, or 15 mg furan/kg (59-220 μmol/kg), this increase was not statistically significant (~15, ~18, and ~19, respectively, vs. ~12.0 in controls). However, when the control groups for the 3-wk exposure study and the 4-wk exposure study were pooled, a statistically significant increase in the hepatocyte labeling index occurred in animals treated with 8 or 15 mg furan/kg (117 or 220 μmol/kg) as compared to the pooled control group (the labeling indices for the 2 control groups were not significantly different from each other). *second experiment*: Liver: Positive (for proliferative activity, as indicated by labeling index) Liver weight was significantly increased (~1.2-fold) in furan- and furan + ABT-treated animals as compared to controls (data not shown). The hepatocyte labeling index was significantly increased in furan-treated animals (~14 vs. ~10 in controls; p ≤ 0.05). Treatment with furan + ABT completely prevented furan-induced inflammation and necrosis, as well as the elevation in plasma activity of liver enzymes and bile acid levels. However, animals treated with furan + ABT displayed mild hepatocyte vacuolization which was not detected in animals treated with furan alone or corn oil alone.	

Table 6-1. Cell Proliferation Induced By Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
8- to 10-wk-old B6C3F$_1$/CrlBR mouse	5M (for each dose)	5M (corn oil alone)	furan, >99% pure (same lot as that used in NTP [1993] bioassay)	50 mg/kg bw by gavage in corn oil, single dose (734 μmol/kg/day)	single dose	*Single dose:* Animals were killed at various times between 12 hours and 8 days. Two hours before being killed, animals were injected i.p. with 2 mL/kg of methyl[^{3}H]- thymidine (1000 μCi/mL) for labeling index evaluation. **Liver:** Positive (for proliferative activity, as indicated by labeling index) There was a sharp increase in the hepatocyte labeling index in animals 48 hours after treatment with furan (< 0.5 at 12 h, < 0.5 at 24 h, 23.9 at 48 h, ~2 at 4 days, and < 0.5 at 8 days vs. < 0.5 in controls at 48 h).	Wilson et al. (1992) Butterworth et al. (1994) Sprankle et al. (1994) Wilson et al. (1992; cited by IARC, 1995)* * IARC (1995) listed values for hepatocyte labeling index, while Wilson et al. (1992) only provided a graph.
10- to 12-wk-old B6C3F$_1$/CrlBR mouse	6M (for each time point)	6M (corn oil alone)		15 mg/kg bw/day by gavage in corn oil, 5 days/wk (220 μmol/kg/day)	1, 3, or 6 wk	*Repeated doses:* Six days before being killed, animals were implanted s.c. with an osmotic pump containing [^{3}H]thymidine. **Liver:** Positive (for proliferative activity, as indicated by labeling index) There was an increase in the hepatocyte labeling index in animals treated for all time periods (25.1 at wk 1, 12.0 at wk 3, and 3.2 at wk 6 vs. controls values of 0.41 at wk 1 and 0.89 at wk 6). The hepatocyte labeling index for controls receiving treatment for 3 weeks was not determined.	

Table 6-1. Cell Proliferation Induced By Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
51-day-old B6C3F₁ mouse	10M, 10F (for each dose level)	10M, 10F (corn oil alone)	furan, >99% pure	*males*: 2, 4, 8, or 15, or 30 mg/kg bw/day by gavage in corn oil, 5 days/wk (29-441 μmol/kg bw/day) *females*: 4, 8, 15, 30, or 60 mg/kg bw/day by gavage in corn oil, 5 days/wk (59-881 μmol/kg bw/day)	13 wk	Survivors were killed at the end of the 13-week study. Statistical analyses were performed using the Fisher exact test. **Liver:** Positive (for proliferative activity, as indicated by presence of hyperplasia) The incidence of biliary tract hyperplasia was significantly increased in females, but not males, treated with 30 or 60 mg (441 or 881 μmol) furan per kg mean body weight (8/10 and 10/10 vs. 0/10 controls; p ≤ 0.01). The incidence of cholangiofibrosis was significantly increased in females, but not males, treated with 30 or 60 mg (441 or 881 μmol) furan per kg mean body weight (4/10 [p ≤ 0.05] and 10/10 [p ≤ 0.01] vs. 0/10 controls). The incidence of hepatocyte cytomegaly was significantly increased in males administered 30 mg (441 μmol) furan per kg mean body weight (10/10 vs. 0/10 controls; p ≤ 0.01) and in females administered 30 or 60 mg (441 or 881 μmol) furan per kg mean body weight (10/10 and 10/10 vs. 0/10 controls; p ≤ 0.01).	NTP (1993)
58-day-old B6C3F₁ mouse	50M, 50F (for both dose levels)	50M, 50F (corn oil alone)	furan, >99% pure	8 or 15 mg/kg bw/day by gavage in corn oil, 5 days/wk (117 or 220 μmol/kg bw/day)	up to 104 wk	Animals were killed at the end of 2 years. Statistical analyses were performed using logistic regression tests. **Liver:** Positive (for proliferative activity, as indicated by presence of hyperplasia) The incidence of focal hyperplasia was significantly increased in furan-treated animals (44/50 low-dose and 49/50 high-dose males vs. 1/50 male controls [p < 0.001]; 48/50 low-dose and 48/50 high-dose females vs. 0/50 female controls [p < 0.001]). The incidence of biliary tract chronic inflammation was significantly increased in furan-treated animals (44/50 low-dose and 49/50 high-dose males vs. 0/50 male controls [p < 0.001]; 48/50 low-dose and 50/50 high-dose females vs. 2/50 female controls [p < 0.001]). The incidence of biliary tract fibrosis was significantly increased in furan-treated animals (45/50 low-dose and 49/50 high-dose males vs. 0/50 male controls [p< 0.001]; 47/50 low-dose and 50/50 high-dose females vs. 0/50 female controls [p< 0.001]). The incidence of biliary tract hyperplasia was significantly increased in furan-treated animals (46/50 low-dose and 49/50 high-dose males vs. 0/50 male controls [p< 0.001]; 47/50 low-dose and 50/50 high-dose females vs. 0/50 female controls [p < 0.001]). The incidence of hepatocyte cytomegaly was significantly increased in furan-treated animals (45/50 low-dose and 50/50 high-dose males vs. 8/50 male controls [p< 0.001]; 48/50 low-dose and 50/50 high-dose females vs. 0/50 female controls [p< 0.001]). **Adrenal Medulla:** Positive (for proliferative activity, as indicated by presence of hyperplasia) The incidence of focal hyperplasia was significantly increased in males and females treated with the high dose (9/50 high-dose males vs. 0/49 male controls [p = 0.002]; 8/50 high-dose females vs. 2/50 female controls [p = 0.033]).	NTP (1993)

Table 6-1. Cell Proliferation Induced By Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats							
8- to 10-wk-old F344/CrlBR rat 10 to 12-wk-old F344/CrlBR rat	5M (for each treatment) 6M, 6F (for each time point)	5M (corn oil alone) 6M, 6F (corn oil alone)	furan, >99% pure	30 mg/kg bw by gavage in corn oil, single dose (441 µmol/kg bw) 8 mg/kg bw/day by gavage in corn oil, 5 days/wk (117 µmol/kg bw/day)	single dose 1, 3, or 6 wk	*Repeated doses:* **Liver:** Positive (for proliferative activity, as indicated by labeling index) Males and females treated for 6 wk had bile-duct hyperplasia and metaplasia, resembling intestinal cells, in areas of liver fibrosis. The hepatocyte labeling indices in males and females were increased after treatment (males: 3.2 at wk 1, 9.2 at wk 3, and 6.5 at wk 6; females: 11.7 at wk 1, 9.2 at wk 3, and 14.4 at wk 6; controls: 0.08 at wk 1 and 0.29 at wk 6 [males], 0.77 at wk 1 and 0.75 at wk 6 [females]). The hepatocyte labeling index for controls receiving treatment for 3 wk was not determined.	Wilson et al. (1992) Butterworth et al. (1994) Sprankle et al. (1994) Wilson et al. (1992; cited by IARC, 1995)* IARC (1995) listed values for hepatocyte labeling index, while Wilson et al. (1992) only provided a graph.
47-day-old F344/N rat	5M, 5F (for each dose level)	5M, 5F (corn oil alone)	furan >99% pure	*males*: 5, 10, 20, 40, or 80 mg/kg bw/day by gavage in corn oil, 5 days/wk (73-1175 µmol/kg bw/day) *females*: 10, 20, 40, 80, or 160 mg/kg bw/day by gavage in corn oil, 5 days/wk (147-2350 µmol/kg bw/day)	12 days	Animals were killed at the end of the treatment period. **Liver:** Positive (for proliferative activity, as indicated by enlarged livers) Males treated with 20, 40, or 80 mg/kg (294☐1175 µmol/kg/day) and females treated with 40, 80, or 160 mg/kg (588☐2350 µmol/kg/day) had mottled and enlarged livers.	NTP (1993)

Table 6-1. Cell Proliferation Induced By Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
young adult F344 rat	14M	0	furan, (>99% pure)	60 mg/kg bw/day by gavage in corn oil, 5 days/wk (881 µmol/kg bw/day)	10-14 days	Only 4 of 14 animals survived the 10- to 14-day treatment period. **Liver:** Atrophy of the right liver lobe was associated with the appearance of cholangiolarlike structures composed of biliary epithelial cells and usually a single ductular hepatocytic cell in various stages of maturation. Unlike normal cholangioles, the nuclear dimensions and phenotypic features of these structures were consistent with a differentiation of rare bile ductularlike cells to transitional ductular cells to more mature ductular hepatocytes.	Sirica et al. (1994b)
young adult F344 rat	5M (for each dose) 5M	5M (corn oil alone) 5M (corn oil alone)	furan, >99% pure	15, 30, 45, or 60 mg/kg bw/day by gavage in corn oil, 5 days/wk (220-881 µmol/kg bw/day) 60 mg/kg bw/day by gavage in corn oil, 5 days/wk (8881 µmol/kg bw/day)	3 wk 1-2 wk	There was a dose-dependent increase in furan toxicity, with none of the rats treated with 60 mg/kg surviving past the end of the second week of treatment. **Liver:** Positive (for proliferative activity, as indicated by presence of hyperplasia) Animals treated with 45 or 60 mg/kg (661 or 881 µmol/kg) rapidly developed cholangiofibrosis, which was localized to the caudate lobe. The caudate lobe was characterized by the presence of well-differentiated hyperplastic bile ductules and of metaplasia resembling metaplastic intestinal glands supported by a fibrous connective tissue stroma. Other liver lobes (i.e., median and left lateral) were characterized mainly by cirrhosis and areas of hepatocellular degeneration and necrosis (detected sporadically within Zones 2 and 3 of the liver acini).	Elmore and Sirica (1991)
young adult F344 rat	3-4M (for each time point)	0	furan, >99% pure	45 mg/kg bw/day by gavage in corn oil, 5 days/wk (661 µmol/kg bw/day)	1, 3, 5, 7, 9, 12, 16, or 32 days	**Liver:** Positive (for proliferative activity, as indicated by cholangiofibrosis) On day 7, prominent bile ductule hyperplasia was observed in tissue sections of the right liver lobe (and to a lesser extent in the caudate lobe) exhibiting marked loss of normal liver parenchyma. There was a sharp decreased weight in the right liver lobe during treatment from days 9 to 32. This decreased weight corresponded to a sharp rise in the incidence of cholangiofibrosis. By day 32, much of the right lobe had been replaced with cholangiofibrotic tissue. This effect was much less pronounced in the caudate lobe of the liver.	Elmore and Sirica (1992)
young adult F344 rat	"groups of" 3-6M	3-6M (furan controls) 3-6M (bile duct ligation controls)	furan, >99% pure	45 mg/kg bw/day by gavage in corn oil, 5 days/wk (661 µmol/kg bw/day)	5-6 wk	Bile duct ligations were performed one week prior to furan treatment. Controls were ligated and dosed with corn oil or received a sham operation. **Liver:** Furan-treated animals that recieved bile duct ligations had 72.6 ± 16.3% of the liver replaced by well-differentiated hyperplastic bile ductules and had greatly increased activities of γ-glutamyl transpeptidase in liver homogenates. The livers of ligated controls that were dosed with corn oil had 20.0 ± 4.2% bile-duct tissue. The livers of animals that received furan treatment after a sham operation had 11.9 ± 3.1% bile-duct tissue.	Sirica et al. (1994a)

Table 6-1. Cell Proliferation Induced By Furan (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
51-day-old F344/N rat	10M, 10F (for each dose)	10M, 10F (corn oil alone)	furan, >99% pure	4, 8, 15, 30, or 60 mg/kg bw/day by gavage in corn oil, 5 days/wk (59-881 µmol/kg bw/day)	13 wk	Statistical analyses were performed using the Fisher exact test. **Liver:** Positive (for proliferative activity, as indicated by presence of hyperplasia) The incidence of biliary tract hyperplasia was significantly increased in males and females treated with any of the doses (males, in order of increasing dose: 4/10 [p ≤ 0.05], 9/10, 10/10, 10/10, 10/10 [p ≤ 0.01] vs. 0/10 male controls; females, in order of increasing dose: 7/10, 10/10, 10/10, 10/10, 9/10 [p ≤ 0.01] vs. 0/10 female controls).	NTP (1993)
6-wk-old F344 rat	50M	0	furan, purity not specified	30 mg/kg bw/day by gavage in corn oil (5 mL/kg), 5 days/wk (441 µmol/kg bw/day)	13 wk	Animals were killed at the end of treatment, after 9 months, or after 15 months. **Liver:** Positive (for proliferative activity, as indicated by presence of hyperplasia) Animals killed at 90 days had enlarged livers with areas of fibrosis and cellular hyperplasia. Animals killed after 9 and 15 months had similar (in terms of severity and extent) liver abnormalities. However, these abnormalities were more severe than those seen in animals treated with 8 mg/kg bw (117 µmol/kg) for 9 or 15 months.	Maronpot et al. (1991) NTP (1993)

Abbreviations: bw = body weight; wk = week(s); mo = month(s); M = male; F = female

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APPENDIX A

DESCRIPTION OF ONLINE LITERATURE SEARCHES FOR FURAN

DESCRIPTION OF ONLINE LITERATURE SEARCHES FOR FURAN
(IARC Monograph in Vol. 63, 1995)

The searches described below were conducted between March and October 1996. An exhaustive search of all pertinent databases was not attempted, but the ones chosen were expected to provide citations for most of the relevant recently published literature. No attempt was made to find toxicity information about metabolites and other structural analogs in the search strategy.

Ordinarily, if an IARC monograph or another authoritative review has been published, literature searches were generally restricted from the year before publication to the current year. However, because this stratagem was not formulated before the furan toxicity literature searches, the searches were not restricted by publication year; i.e., searches were run against the full databases and were only limited by the years of coverage by each database.

Older literature that needed to be examined was identified from the reviews and original articles as they were acquired. Current awareness was maintained by weekly searches of Current Contents on Diskette[7] Life Sciences 1200 [journals] edition. Dr. Robert Maronpot supplied a recent article and an article that will be in press or published by the time the background document is finalized.

TOXLINE (1940s to date): The contractor printed the titles of the 156 records indexed by furan's Chemical Abstracts Service Registry Number (CASRN) 110-00-9. From these titles, the contractor selected 66 records, printed their citations, and chose 22 citations for immediate retrieval. The subjects of these studies included carcinogenesis, genetic toxicity, oncogene expression in tumors, and other information that might be relevant to its mechanism of carcinogenesis. The other 44 citations were primarily on environmental fate and occurrence, exposure issues, and QSAR. A large number of records in this and other databases that have been indexed by 110-00-9 and/or furan by name are really on polychlorinated dibenzofurans (PCDFs). After reviewing the record titles and citations, Dr. John Bucher selected an additional 3 records for retrieval.

CANCERLIT (1963 to date): Of the 11 records retrieved by use of the CASRN, a 1990 dissertation by Y. Wang on chemically induced and spontaneously arising activation of *ras* oncogenes was unique to this database.

EMBASE (1974 to date): EMBASE is the online version of Excerpta Medica, which is similar to Index Medicus/MEDLINE; the database has good coverage of environmental issues as well as toxicity. The contractor examined titles of 126 records indexed by 110-00-9 and identified 14 citations of interest not found among the TOXLINE or TOXLIT titles.

EMIC (Environmental Mutagen Information Center) (1991 to date); EMICBACK (1950 to 1990): Fifteen records were indexed by furan's CASRN.

<u>IRIS</u>: The furan profile found in March 1996 did not include a carcinogenicity risk assessment.

<u>NTIS</u> (1964 to date): This database of government reports is a good place to identify health effects and exposure assessment reviews. Seven records were indexed by 110-00-9: 1 was the NTP bioassay, 1 was on controlling accidental releases, and 5 represented a multi-volume NIOSH report written in 1984. The latter included a negative hamster carcinogenesis bioassay of fumes emitted from furan resins used for casting molds in iron foundries. This study has been published in a journal. Since no reference that reliably identifies furan as a constituent of these fumes was found, this study was not included in the review. AFuran resins@ are generally produced from furfuryl alcohol.

<u>Occupational Safety and Health (NIOSHTIC)</u> (1973 to date): The search statement was RN=110-00-9 [41 records] OR (furan not (dioxin? or furans)) [82 records]. Among the titles examined were 13 records already selected from other databases, 19 records obviously about other furans, and 17 records related to exposure assessment.

<u>TOXLIT</u> (1965 to date): TOXLIT (Toxicology Literature from Special Sources) is an NLM-produced database from records supplied by CAS. The CAS records have been selected from portions of Chemical Abstracts that cover pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals. Although thoroughly indexed by CASRN, the database shares the problem of inappropriate records on PCDFs having been indexed by 110-00-9. The titles of 297 records indexed by the CASRN were examined; 53 records appeared to be of interest. These records were compared with TOXLINE and EMBASE retrievals and 26 new citations were selected for acquisition.

<u>TSCATS</u> (early 1980s to date): The EPA Toxic Substances Control Act Test Submissions database was searched on the Chemical Information System with furan's CASRN. The two reports found were entitled, AAcquisition and chemical analysis of mother's milk for selected toxic substances (December 1980), done for EPA by Research Triangle Institute, and AInitial submission: Mortality patterns of workers in the Niagara plant (Final report on mixtures of chemical substances) with attachments and cover letter dated 022192,@ performed by the University of Pittsburgh for Occidental Chemical Corporation.

In September 1996, the contractor performed searches for updating sections 1 and 2, which had been last updated in 1994 with regulatory information from print sources and REGMAT (May 1993 version). REGMAT had broad coverage of EPA regulations, but it is no longer available. Databases searched in 1996 included CSCHEM and CSCORP for U.S. suppliers (databases produced by Chem Sources); HSDB; the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System) and ISHOW (for physical-chemical properties); Chemical Abstracts Service's (CAS) File CHEMLIST for TSCA and SARA updates in 1996; and CAS's CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information.

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test

Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test

Code	**Definition**
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange